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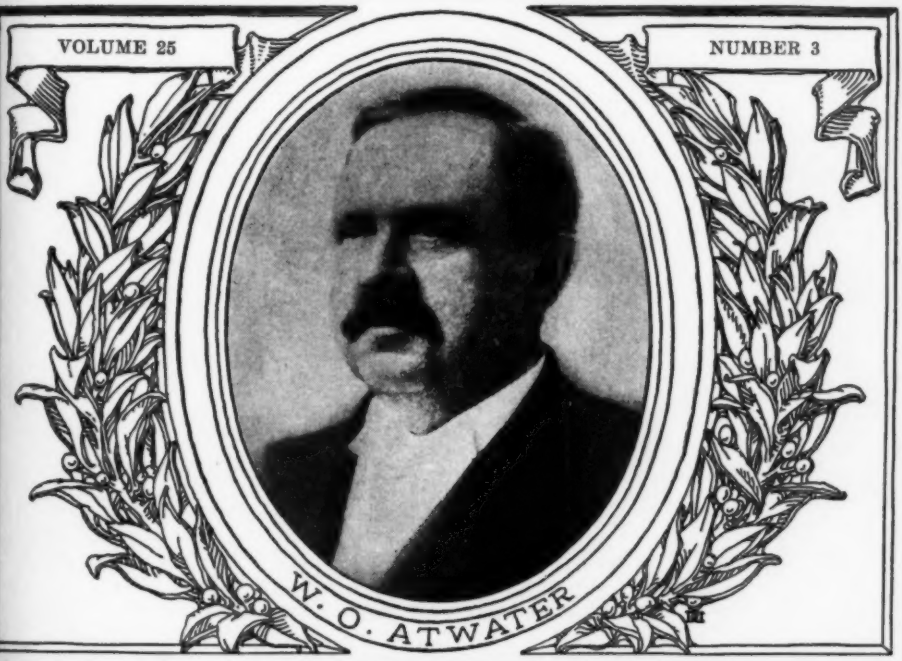

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FURTHER STUDIES ON RIBOFLAVIN AND THIAMINE IN THE RUMEN CONTENT OF CATTLE. II[1]

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(Received for publication September 3, 1942)

In a previous report (Hunt et al., '41) it was shown that riboflavin was synthesized in the rumen of the steer. There was also an indication that when the corn content of the ration was the same (by weight) as the roughage, the ration containing whole corn produced less riboflavin than the ration containing the same amount of ground corn. The results also showed that the thiamine content of the dried ingesta 4 hours after feeding was slightly greater than that of the feed, whereas 12 to 16 hours after feeding the thiamine content of the dried ingesta was lower than that of the feed.

McElroy and Goss ('39, '40) have shown that several factors of the vitamin B complex, including riboflavin and thiamine, are synthesized in the rumen of the sheep and the cow, although in one experiment (McElroy and Goss, '41) no thiamine was detected in the ingesta of a cow fed a partially synthetic low vitamin B complex ration. Wegner, Booth, Elvehjem and Hart ('40, '41) also reported the synthesis of several members of the vitamin B complex in the rumen of cattle and stated that there was no evidence indicating that thiamine was destroyed in the rumen.

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The present studies were undertaken to obtain further information on the effects on the synthesis of riboflavin and thiamine in the rumen of (a) whole versus ground corn, (b) varying proportions of hay to corn, and (c) increasing amounts of fairly pure carbohydrates in a partially synthetic ration.

EXPERIMENTAL

The data reported herein are from three separate experiments. In experiment 1, the daily ration consisted of 7.5 pounds of yellow corn, 3.5 pounds of alfalfa hay, and 1.5 pounds of a protein supplement. The corn was fed whole in one period and ground in a second period. Each ration was fed to two steers with a rumen fistula. The daily rations fed in experiment 2 contained a variable amount of hay and whole yellow corn with a constant amount of a protein supplement (table 1). Three steers with rumen fistula were used in this

TABLE 1

Steer rations as fed daily (experiment 2).

FEED	PERIOD 1 FOR STEER NO.			PERIOD 2 FOR STEER NO.			PERIOD 3 FOR STEER NO.		
	5	6	7	5	6	7	5	6	7
	lb.	lb.	lb.	lb.	lb.	lb.	lb.	lb.	lb.
Hay	10.5	5.25	2	2	8.5	4.25	4.25	2	8.5
Protein supplement	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Corn	.0	5.25	8.5	6.5	.0	4.25	4.25	6.5	.0

experiment. In experiment 3, partially synthetic rations with varying amounts of carbohydrates were fed to the same three steers used in experiment 2. The composition of the rations is shown in table 2.

All rations were fed for 10 to 14 days before samples of ingesta were collected. In collecting these samples the entire rumen content was removed from each steer, mixed thoroughly and sampled; the remainder was returned to the rumen. The pH of the rumen content was determined at the time of collection. The freshly sampled ingesta were treated with 95% ethanol so that the final concentration was about 40%, by

TABLE 2

Percentage composition of steer rations fed daily (experiment 3).

SUPPLEMENTS	RATION		
	1	2	3
	%	%	%
Beet pulp (dried)	57	39	21
Soybean oil meal (solvent)	20	24	28
Cellulose (regenerated)	19	13	7
Wheat starch	0	10	20
Corn sugar (cerelose)	0	10	20
Salt mixture { 1 part NaCl, 2 parts bonemeal, 2 parts $CaCO_3$	4	4	4

volume, made acid with HCl, and stored in a cold dark room until ready for drying. The sampling of the feed, and the drying and grinding of the ingesta were the same as described in a previous report (Hunt et al., '41). All samples represented a 3-day collection.

The rat assay method was used for all riboflavin and thiamine studies in experiment 1. The experimental procedure, rations used, and supplements fed in these assays were the same as those described in previous reports (Hunt and Bethke, '40; Hunt et al., '41). Calibrated or reference curves were obtained by feeding different levels of either pure riboflavin or thiamine [2] which were used to estimate the riboflavin and thiamine content of the feeds and ingesta. The results are shown in table 3.

The microbiological method of Snell and Strong ('39) was used for the riboflavin assays in experiments 2 and 3. These results are shown in tables 4, 5, and 7, respectively. There are also included in table 5 the results of the rat assays on the feed and the composite samples of the ingesta from the three steers on each feed. The procedure employed in the rat assay was similar to that used in experiment 1 except that 100 μg. of pantothenic acid as calcium pantothenate, 3 mg. of choline hydrochloride, 40 μg. of pyridoxine and 0.5 mg. of

[2] Merck's.

TABLE 3

Growth rate of rats: weight increase on varying amounts of riboflavin and thiamine, respectively.

SUPPLEMENT	NUMBER OF ANIMALS	AMOUNT FED DAILY	AVERAGE GAIN PER WEEK	AVERAGE GAIN OVER CONTROLS PER WEEK
		μg.	*gm.*	*gm.*
Riboflavin	10	5.0	8.3	7.5
Riboflavin	10	10.0	13.0	12.2
Riboflavin	10	20.0	18.6	17.8
Negative control	8	0.0	0.8	
Thiamine	8	2.0	4.0	4.0
Thiamine	8	4.0	10.0	10.0
Thiamine	8	6.0	14.0	14.0
Negative control	8	0.0	—0.75	

TABLE 4

The riboflavin[1] and thiamine[1] content of the feed and ingesta (experiment 1).

DISTINGUISHING FEATURE OF SAMPLE ASSAYED	TIME AFTER LAST FEEDING	AVERAGE GAIN OVER CONTROLS PER GRAM OF SAMPLE PER WEEK		ESTIMATED CONTENT PER GRAM	
		Riboflavin-free ration[2]	Thiamine-free ration	Riboflavin	Thiamine
	hr.	*gm.*	*gm.*	*μg.*	*μg.*
Feed		4.7	9.2	6.2	3.8
Ingesta [3] Ground corn	5	11.0	10.8	17.4	4.9
Ingesta [3] Ground corn	16	13.7	11.1	25.0	5.0
Ingesta [3] Whole corn	5	9.2	8.8	14.0	3.7
Ingesta [3] Whole corn	16	12.2	9.0	20.0	3.8

[1] Rat assay (biological) method.

[2] 0.5 gm. sample.

[3] The feed and ingesta assayed were composite samples from two steers.

TABLE 5

The riboflavin content of the feed and ingesta (experiment 2).

DISTINGUISHING FEATURE OF RATIONS ASSAYED	FEED		TIME AFTER LAST FEEDING	STEER 5		STEER 6		STEER 7		COMPOSITE INGESTA — ALL STEERS
	1	2		Ingesta		Ingesta		Ingesta		
	μg./gm.			μg./gm.[1]	pH[2]	μg./gm.[1]	pH[2]	μg./gm.[1]	pH[2]	μg./gm.[3]
High hay	12.7	11.2	4	10.0	6.6	11.3	6.8	10.1	6.6	12.5
No corn			16	8.0	7.4	10.0	7.4	12.2	7.3	11.0
Medium hay	10.5	9.8	4	11.9	6.3	12.9	6.1	10.5	6.5	11.2
Medium corn			16	15.5	6.9	16.8	6.9	12.0	7.2	17.5
Low hay	5.7	6.6	4	19.5	6.3	20.5	6.2	19.1	6.3	
High corn			16	21.0	6.8	24.0	6.8	21.5	7.0	

[1] Microbiological method.

[2] Average of two samples.

[3] Rat assay (biological method).

niacin daily were substituted for the yeast filtrate fraction (Hunt et al., '41).

The thiamine content of the feed and ingesta samples from experiments 2 and 3 was determined by the rat assay method. The procedure was the same as used in experiment 1 (Hunt et al., '41) except that 100 μg. of pantothenic acid, 3 mg. of choline hydrochloride, 40 μg. of riboflavin, 40 μg. of pyridoxine, and 0.5 mg. of niacin daily were substituted in place of autoclaved yeast or yeast filtrate fraction. These results for experiments 2 and 3 are recorded in tables 6 and 7, respectively.

DISCUSSION OF RESULTS

The results of the three experiments (tables 4, 5, and 7) show that riboflavin is synthesized in the rumen, a finding which is in accord with the authors' previous observations (Hunt et al., '41) and those of McElroy and Goss ('39, '40, '41) and Wegner et al. ('40, '41). It is also apparent that the kind and state of the feed consumed are factors in the synthesis of riboflavin in the rumen. Ground yellow corn

appeared to effect a larger concentration of riboflavin in the rumen content than an equal amount of whole corn (table 4). Increasing the amount of corn in the ration also caused an increased riboflavin content of the ingesta (experiment 2, table 5). Presumably the inclusion of corn in the ration provided a more favorable medium, by supplying available carbohydrates for the growth of the bacteria involved in riboflavin synthesis.

TABLE 6

The thiamine content[1] of the feed and ingesta (experiment 2).

DISTINGUISHING FEATURE OF RATIONS & INGESTA	TIME AFTER LAST FEEDING	NUMBER OF RATS	AMOUNT FED DAILY	AVERAGE GAIN OVER CONTROLS PER WEEK	ESTIMATED THIAMINE CONTENT PER GRAM
	hr.		*gm.*	*gm.*	*μg.*
High hay No corn[2]		8	1.0	4.4	2.5
Medium hay Medium corn[2]		8	1.0	7.1	3.5
Low hay High corn[2]		8	1.0	8.0	4.0
Ingesta[2] High hay No corn	4	8	1.0	.56	>.2
Ingesta[2] High hay No corn	16	8	1.0	0.00	All died of polyneuritis
Ingesta[2] Medium hay Medium corn	4	8	1.0	2.7	1.5
Ingesta[2] Medium hay Medium corn	16	8	1.0	2.1	1.0
Ingesta[2] Low hay High corn	4	8	1.0	8.2	4.0
Ingesta[2] Low hay High corn	16	8	1.0	5.8	3.0

[1] Rat assay (biological) method.

[2] All rations and ingesta assayed were composite samples from three steers.

This assumption is borne out by the fact that in the corn-free ration (experiment 2, table 5) there was but little, if any, synthesis of riboflavin in the rumen as judged by the comparative riboflavin content of the feed and the dried ingesta. Similar observations were noted in our earlier investigations (Hunt et al., '41).

The difference in the riboflavin content of the ingesta from whole corn and ground corn rations may be partially due to

TABLE 7

The riboflavin[1] and thiamine[2] content of the feed and ingesta (experiment 3).

DISTINGUISHING FEATURE OF SAMPLES ASSAYED	FEED		TIME AFTER LAST FEEDING	STEER 5		STEER 6		STEER 7		COMPOSITE INGESTA — ALL STEERS THIAMINE
				Ingesta		Ingesta		Ingesta		
	Riboflavin	Thiamine		Riboflavin	pH	Riboflavin	pH	Riboflavin	pH	
	μg./gm.			*μg./gm.*		*μg./gm.*		*μg./gm.*		*μg./gm.*
Ration 1	1.2	<.2	4	10.7	6.2	16.6	6.0	19.4	6.1	All dead at end of 3 weeks
Low carbohydrates			16	11.8	7.1	21.3	7.1	21.8	7.0	All dead at end of 3 weeks
Ration 2	1.9	<.2	4	19.3	6.2	17.0	6.1	22.6	6.1	All dead at end of 3 weeks
Medium carbohydrates			16	24.3	7.2	18.8	7.3	30.3	7.1	All dead at end of 3 weeks
Ration 3	3.4	0.6	4	14.8	5.7	24.4	5.9	16.3	5.9	0.3
High carbohydrates			16	21.6	6.8	32.5	7.1	29.5	7.1	60% alive at end of 3 weeks

[1] Microbiological method.

[2] Rat assay (biological) method.

the greater concentration of water soluble material per gram of rumen dry matter in the case of the ground corn, because it has been shown (unpublished data) that ground corn leaves the rumen somewhat faster than whole corn. This probable difference in concentration of water soluble material per unit of dry matter, however, cannot account for the severalfold increase in the riboflavin of the dried rumen content over that of the feed.

The data do not show any definite correlation between the pH and riboflavin content of the dried ingesta. All of the 16-hour ingesta samples from the corn-free, hay, and protein supplement ration were alkaline, and alkalinity might be considered unfavorable to the stability of riboflavin. However, some of the other ingesta samples (tables 5 and 7) had a pH of 7 or above and still showed a high riboflavin content. All ingesta samples taken 4 hours after feeding had a pH of 6.9 or less which, in all cases, became less acid as the period after feeding increased, as shown by the pH results taken at the 16-hour period. It accordingly would appear that the carbohydrate content of the ration was more of a determining factor in the synthesis of riboflavin in the rumen than was the pH of the rumen content.

The results with the partially synthetic ration containing different amounts of carbohydrates are variable between animals (table 7). Nevertheless, the results in general indicate that the higher the carbohydrate content of the rations the greater the riboflavin content of the dried ingesta. These results confirm the observations with corn (table 5).

It should be noted that after the experiment with the partially synthetic rations was started it was discovered that the beet pulp contained some added molasses, which made ration 1 higher in total carbohydrates than was originally intended and also caused a somewhat less variation in carbohydrate content between rations.

The results of the rat (biological) assay of the feeds and composite ingesta samples (table 5) compare very favorably with the results obtained by the microbiological method.

Data for the thiamine assays are presented in tables 4, 6, and 7. It will be observed (table 4) that when ground corn was fed, the thiamine content of the ingesta was slightly higher than that of the feed. This may indicate that the amount of corn (source of carbohydrates) and its fineness influenced the amount synthesized. Another possible explanation is that ground corn passes out of the rumen at a faster rate and yet leaves behind a greater amount of soluble material per gram of dry matter than whole corn (unpublished data). This fact would tend to concentrate the thiamine in the remaining rumen content or ingesta. The results with whole corn (source of carbohydrates) show that thiamine is not synthesized (tables 4 and 6) when rations of the type used in these experiments are fed. On the contrary, there is an actual loss, but this loss decreased as the amount of corn (source of carbohydrates) in the ration was increased. The results obtained from feeding the partially synthetic low-vitamin feed show a similar loss of thiamine in the ingesta below that of the feed, but this loss decreased slightly as the amount of carbohydrates in the feed was increased (table 7). These observations on thiamine are in accord with the authors' previous findings (Hunt et al., '41) but contrary to some of the reports of McElroy and Goss ('39, '40) and Wegner, Booth, Elvehjem and Hart ('40, '41). They do not necessarily preclude the synthesis of thiamine in the rumen. However, if synthesis did occur, the thiamine was rapidly absorbed or destroyed through bacterial action. There is also the possibility that some loss occurred during the preparation of the samples.

SUMMARY AND CONCLUSIONS

The data presented show that riboflavin is synthesized in the rumen of the steer when a ration containing corn, alfalfa hay, and a protein supplement is fed.

When corn was omitted from the alfalfa hay-protein supplement rations there was no apparent synthesis of riboflavin as judged by the comparative riboflavin content of the ration

and the dried ingesta. As the amount of corn in the ration increased, there was a corresponding increase in the riboflavin content of the ingesta. Additional evidence was obtained that when ground corn was fed, the riboflavin content of the ingesta was higher than that of the ingesta from a similar ration containing an equal weight of whole corn.

The results with a partially synthetic low-vitamin ration also show that riboflavin is synthesized in the rumen and that there appears to be a correlation between the amount of carbohydrates in the ration and the riboflavin content of the dried ingesta.

There was no evidence obtained that thiamine was synthesized in any of the experiments as judged by the comparative thiamine content of the feeds and the dried ingesta, with the possible exception of the one in which ground corn was fed. The results with both whole corn as part of the ration and with the partially synthetic low-vitamin ration show that there was a loss of thiamine in the ingesta in comparison with the feed, but the loss incurred decreased as the amount of corn or carbohydrates in the rations increased.

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VITAMIN B COMPLEX STUDIES IN DOGS: PRODUCTION OF CIRRHOSIS OF LIVER

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TWO FIGURES

(Received for publication August 10, 1942)

Fouts, Helmer and Lepkovsky ('40) found that adult dogs fed a synthetic casein (41%) diet supplemented with thiamine chloride, riboflavin, nicotinic acid and pyridoxine developed a deficiency state characterized by loss of appetite, marked loss of weight, intermittent diarrhea, moderate anemia and death. The livers of these animals appeared fatty, and in some there were ulcers in the gastrointestinal tract and skin. These findings resembled those produced by Chick, Macrae, Martin and Martin ('38) in pigs fed a synthetic diet similarly supplemented. The central nervous system involvement reported by Chick et al. ('38), and Wintrobe, Mitchell and Kolk ('38) in pigs, and by Phillips and Engel ('39) in chicks, was not observed. The purpose of the present experiments was to continue the study of this deficiency state.

METHODS

The high protein diet used had the following percentage composition: washed casein 41.4, sucrose 29.6, hydrogenated cottonseed oil [1] 25.7, bone ash 2.0, salt mixture no. 185 (McCollum and Simmonds, '18) 1.3 gm. The low protein diet contained: washed casein 15 gm., sucrose 51, hydrogenated cottonseed oil [1] 28, salt mixture no. 185 4, and cod liver oil 2.

[1] Crisco.

The dogs were allowed to eat as much of the respective diets as desired. The supplements were fed by dropper daily in amounts of: thiamine chloride, riboflavin and pyridoxine hydrochloride 60 μg. each per kilo, calcium pantothenate 0.6 mg. and nicotinic acid 2 mg. per kilo, and 8 drops of fish liver oil containing 8000 USP units vitamin A and 1600 USP units vitamin D. The supplements just mentioned will be referred to hereafter as the basic supplements. The amounts of choline, para-aminobenzoic acid, and inositol administered to the dogs are recorded in the text. The filtrate from fullers' earth and charcoal absorption of rice bran extract was similar to the filtrate fraction used by Dimick and Lepp ('40) and probably contained negligible quantities of choline. Red blood cell counts, hemoglobin determinations (Evelyn), and white blood cell counts were made at weekly intervals. Hematocrit measurements were made by the use of Wintrobe tubes.

RESULTS

Puppies nos. 4, 5 and 6 received the high protein diet supplemented with the basic supplements of thiamine, riboflavin, pyridoxine, nicotinic acid and pantothenic acid in addition to choline 10 mg. per kilo. They gained 3.2, 6.2 and 8.3 kg. during the 598, 414 and 414 days on the diet respectively, and developed no signs of disease during this time. On reducing the amount of casein in the diet to 15% dogs nos. 5 and 6 showed rapid weight loss and developed skin ulcers. Their subsequent course will be discussed later. Puppies nos. 1, 2 and 3 received the high protein diet and all the supplements mentioned above except pantothenic acid and choline. They lost weight rapidly, had intermittent diarrhea and died after 39, 86 and 109 days on the diet respectively. All three showed fatty cirrhotic livers. No. 2 had a penetrating ulcer in the pyloric region and no. 3 had a perforated ulcer in the first portion of the duodenum. These findings are similar to those reported previously (Fouts, Helmer and Lepkovsky, '40).

Dogs nos. 7, 8, 9, 11, 12 and 15 were fed the low protein diet and the basic supplements. They all lost weight, developed

skin ulcers and died within from 88 to 267 (average 175) days. The inclusion of 0.5% cystine in the diet of nos. 7 and 8, the administration of 80 mg. of para-aminobenzoic acid per kilo during the last 19 days to dog no. 9, and 20 mg. choline per kilo for 17 days before the death of no. 8 did not seem to influence the course of the deficiency. Dog no. 12 received 10 mg. choline per kilo from the beginning of the study. After 190 days on the experiment 100 mg. inositol per day, 40 mg. para-aminobenzoic acid per kilo, and an eluate of clay absorption of liver extract were successively added to the basic supplements without preventing death on the 225th day. Dog no. 15 received 40 mg. para-aminobenzoic acid, 10 mg. choline per kilo and 100 mg. inositol per day throughout the studies, with little or no influence on the development of the deficiency. All of these dogs showed fatty cirrhotic livers and ulcers of the skin. Dogs nos. 7, 9, 11 and 12 had chronic peptic ulcers. The anemia in all of these animals was of moderate degree except for a terminal fall to a red blood cell count of 2.66 million and hemoglobin of 6.1 gm. in dog no. 8.

Dog no. 10, after 259 days on the low protein diet and basic supplements, received 10 gm. of powdered liver extract and 10 mg. choline per kilo daily. During the 85 days of liver extract administration the skin ulcers and conjunctivitis which had developed disappeared and the dog gained 1.2 kg. in weight. One hundred seventy-four days after discontinuance of the liver extract the conjunctivitis and skin ulcers returned. Increasing the choline to 100 mg. per kilo did not prevent death 12 days later. There was a precipitous drop in red blood cell count to 2.48 million and hemoglobin to 5.3 gm. during the last week of life. At autopsy a ruptured duodenal ulcer was found as well as very extensive fatty cirrhotic changes in the liver. The normal architecture of liver was completely destroyed and there were small white fatty tumors scattered through the liver.

Dog no. 18, because of refusal to eat the low protein diet, received the basic supplements alone for only 20 days. On

receiving 10 gm. of powdered liver daily, food consumption increased and there was a steady increase in weight. When the experiment was terminated on the 140th day, no skin or peptic ulcers were present. The liver weighed 566 gm. and was quite fatty.

Dogs nos. 13 and 14 received 40 mg. para-aminobenzoic acid and 10 mg. choline in addition to the basic supplements and the low protein diet. On the 153rd day 100 mg. inositol per day was added to the supplements given dog no. 13. There was a temporary gain in weight but the extensive skin ulcers increased in size and number. The administration of 40 cc. of the filtrate from a fullers' earth, charcoal adsorbate of rice bran extract was followed by the rapid disappearance of skin ulcers and a gain in weight of 2.7 kg. The weight had decreased 1.6 kg., however, by the 329th day when the dog died following the accidental aspiration of supplements. At autopsy the liver showed marked fibrosis, fatty infiltration and atrophy of liver cells. Dog no. 14 responded with a gain in body weight and disappearance of skin ulcers when 10 gm. of powdered liver extract was added to the supplements on the 195th day. There was a further slight gain in weight when 100 gm. per kilo of choline was administered after the weight became stationary 115 days later.

The low protein diet fed dogs nos. 16 and 17 was supplemented with 100 mg. choline per kilo in addition to the basic supplements. No signs of deficiency developed in dog no. 16 during 92 days on the diet, but on termination of the experiment it was found that the liver was quite fatty and slight fibrosis was present. Dog no. 17, after a preliminary gain in weight, lost 2.4 kg. and developed numerous dermal ulcers. When the experiment was terminated on the 92nd day, the liver of this animal showed increased amount of fat and beginning fibrosis.

Two hundred ninety-three days after reducing the amount of casein in the diet to 15%, dog no. 5 had lost 3.0 kg. in body weight and several skin ulcers were observed. The addition

to the supplements of 10 gm. of powdered liver extract per day produced a gain in weight of 0.8 kg. after 23 days but the skin ulcers increased in number. Increasing the amount of choline to 100 mg. per kilo was followed by more rapid gain in weight and disappearance of skin ulcers. The dog had gained 3.5 kg. when killed 97 days later. The liver weighed 484 gm. and was coarsely nodular. It was still slightly fatty, and fibrosis was extensive.

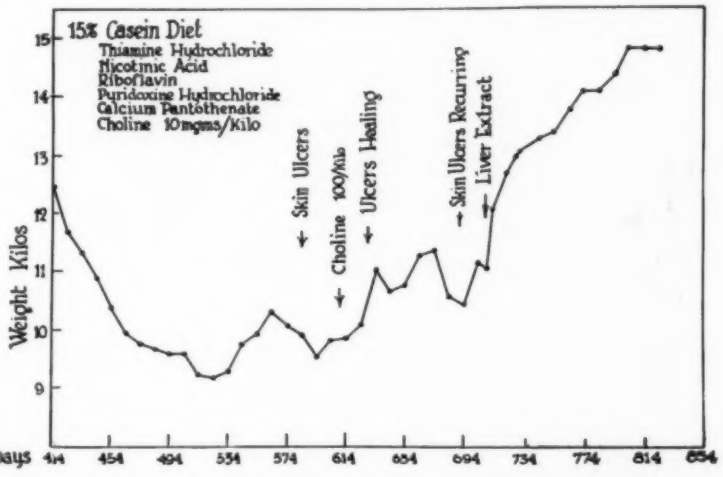

Fig. 1 Weight of dog no. 6 after reduction of amount of casein in diet to 15%, showing the response to increased amounts of choline and to powdered liver extract (10 gm. per day).

As seen in figure 1, dog no. 6 lost weight rapidly on reducing the amount of casein in the diet. The response to 100 mg. of choline per kilo was definite but recovery was not complete. The administration of 10 gm. of powdered liver extract was followed by rapid gain in weight and the healing of ulcers. When the experiment was stopped after 120 days of liver therapy, the liver weighed 603 gm. and was very nodular (fig. 2). It did not appear fatty on a cut surface, but on microscopic examination increased fat in the liver cells was evident.

There was much fibrosis and numerous fatty humors were scattered throughout the liver.

Dogs nos. 19 and 20 received the high protein diet but pyridoxine was not included in the basic supplements although 10 mg. of choline per kilo was added. Both animals developed severe hypochromic, microcytic anemias (red blood cell count

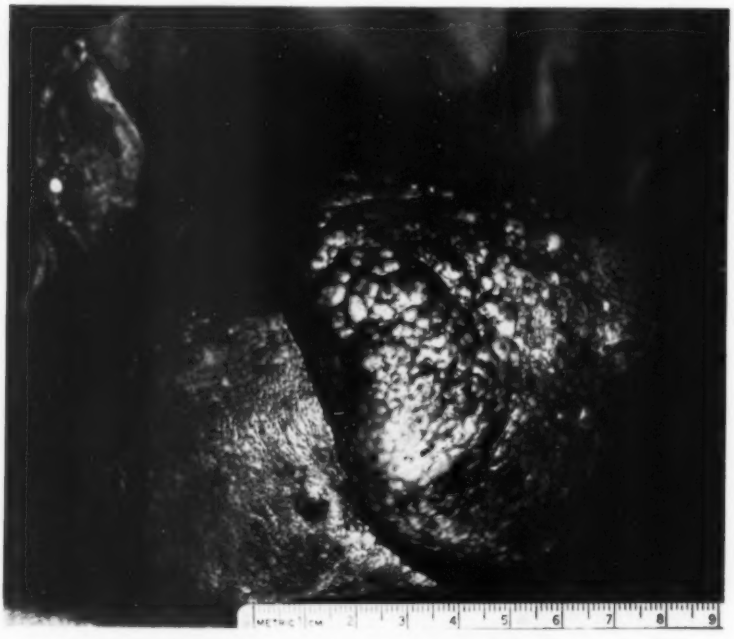

Fig. 2 Liver of dog no. 6, showing marked nodularity of liver after treatment with choline and liver extract.

1.83 and 2.13 million, hemoglobin 2.9 and 4.2 gm., hematocrit 9 and 11.6 cc., respectively). Dog no. 19 had numerous small superficial skin ulcers while no. 20 had many large ulcers of the skin. The death of dog no. 19 occurred on the day pyridoxine therapy was started. The administration of 60 μg. of pyridoxine per kilo was followed by a reticulocytosis of 14.4% and a rapid rise in both red blood cell count and hemoglobin

in dog no. 20. Eighteen days later the red blood cell count was 4.7 million, hemoglobin 11.5 gm., and hematocrit 42 cc., and the ulcers had healed. In spite of a gain of 1.8 kg. during the 32 days following pyridoxine therapy this animal suddenly lost her appetite and died 3 days later. The livers of both dogs appeared fatty after death.

DISCUSSION

The dogs fed the low protein diet supplemented with thiamine, nicotinic acid, riboflavin, pyridoxine and pantothenic acid developed anorexia, loss of weight, anemia, skin ulcers, peptic ulcers, and fatty cirrhotic livers and finally died. The dogs fed the high protein diet similarly supplemented showed suboptimal growth but appeared normal even when fed this diet for long periods of time. The high protein diet, however, was not entirely protective as dogs fed this diet deficient in either pantothenic acid or pyridoxine developed many of the signs and symptoms noted in dogs fed the low protein diet. In addition dogs nos. 5 and 6 gained more weight after relief of the deficiency than they did while being fed the high protein diet.

These results indicate that dogs require supplements other than the well-recognized members of the vitamin B complex. Dimick and Lepp ('40) demonstrated that these supplements did not support maximal growth in rats and chicks; Troescher-Elam and Evans ('41) observed the same in mice, Emerson and Evans ('41) in rats, and McKibbin, Madden, Black and Elvehjem ('39), and McKibbin, Black and Elvehjem ('40) in dogs.

The studies of Griffith and Wade ('39) and Du Vigneaud, Chandler, Moyer and Keppel ('39) showed the importance of choline in nutrition of rats. Choline was therefore added to the supplements of our dogs in doses of 10 and 20 mg. per kilogram body weight. These amounts did not prevent or cure the deficiency state. Schaefer, McKibbin and Elvehjem ('41) reported that dogs showed a distinct gain in weight following

also followed by a gain in weight and improvement of skin ulcers. The combined administration of choline and powdered liver extract, however, produces marked clinical improvement. Even after 120 days of such therapy the fibrosis persists although there has been a decrease in the amount of fat. Nodularity of liver occurred only in four dogs which responded to therapy with partial or complete clinical cure of the deficiency.

CONCLUSIONS

1. A deficiency state characterized by loss of weight, moderate anemia, dermal and peptic ulcers, fatty cirrhotic livers and death, develops in dogs fed a low protein (15% casein) diet supplemented with thiamine, riboflavin, nicotinic acid, pyridoxine and pantothenic acid.

2. Administration of a high protein (41% casein) diet prevents the condition although growth is suboptimal. It is not cured or prevented by para-aminobenzoic acid, inositol, eluate of a clay absorption of liver extract or by small amounts of choline.

3. Partial clinical improvement follows the administration of large amounts of choline, powdered liver extract or a "filtrate factor" prepared from rice bran extract.

4. Combined administration of large amounts of choline and powdered liver extract produced rapid disappearance of the signs of the deficiency although fibrosis of liver persisted.

The author wishes to thank Dr. H. C. Thornton for the preparation of pathological slides and Dr. Sam Lepkovsky for the preparation of the eluate of clay adsorbate of liver extract and the rice bran extract.

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RESULTS OF FEEDING RATS A THIAMINE-LOW DIET OF A TYPE CONSUMED BY HUMAN BEINGS

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ONE FIGURE

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Because of the recent national interest in an adequate dietary regimen, and at the suggestion of Dr. Russell M. Wilder of the staff of the Mayo Clinic, we undertook a study of the effects on growing rats of feeding a human diet composed of foods which commonly appear on American tables and which are known to be deficient in some of the better appreciated fractions of the B-complex. Indeed, typical symptoms of thiamine deficiency developed in human subjects maintained on this diet (Williams and Mason, '41).

The components of the basal diet selected for study are shown in table 1. The diet may be considered a "poor" diet, but it is similar in many respects to that consumed by portions of our population. The proportions of carbohydrate, protein and fat in the basal diet were similar to those ordinarily found in American diets. Vitamins A and D, ferrous sulfate and tricalcium phosphate were added to the basal diet in amounts sufficient to provide not less than 10 international units (I. U.) of vitamin A, 1.0 I. U. of vitamin D, 0.4 mg. of ferrous sulfate and 1.2 mg. of tricalcium phosphate per gram of diet.

Four diets, differing from one another only in the flour from which the bread component was made, were prepared. For convenience of designation these diets were called A, B, C

TABLE 1

Diets used in the study.

DIET	ARTICLE	GRAMS	ARTICLE	GRAMS
"Diet B" Composition: Basal human diet fed to rats	Bread (made of patent flour)	180	Butter	30
	Skim milk powder	20	Corn flakes	15
	Beef roast	75	Potatoes	75
	Cheese	30	Rice	33
	Gelatin	100	Apple jelly	20
	String beans	75	Carrots	50
	Peaches	130	Pears	130
	Sugar	30	Cake	40
	Candy	20	Tricalcium phosphate	0.6
	Ferrous sulfate	0.2	Iodized salt	2.0
	Vitamin A	5,000 I. U.	Vitamin C	80 mg.
			Vitamin D	500 I. U.
	Moist weight, 1.055 gm. Air dried at 65°C. to 450 gm. for consumption by rats			
Analysis of diet B	Carbohydrate (%): 62.1			
	Protein (%): 13.2			
	Fat (%): 10.8			
	Calories per gram: 4.0–4.2			
	Calories from protein (%): 13.2			
	Calories from fat (%): 24.4			
	Calories from carbohydrate (%): 62.4			

		PER GRAM OF DRY DIET	
		Thiamine μg.	Riboflavin μg.
A	Basal diet except that bread component was made from whole wheat flour.....	1.84	3.60
B	Basal diet as described above	0.87	2.50
C	Basal diet in which white flour was restored in content of thiamine to level of whole wheat flour	1.78	2.70
D	Basal diet in which white flour was restored in content of thiamine and riboflavin to level of whole wheat flour	1.66	3.90

and D. In diet A, the bread was prepared with whole wheat flour; in diet B, a patent white flour was used; in diet C, the patent white flour was restored in content of thiamine to the approximate level of whole wheat flour; and in diet D, the patent white flour was restored in content of thiamine [1] and riboflavin[1] to the approximate levels of whole wheat flour. Each diet, when thoroughly mixed, weighed 1,055 gm., and when dried at a temperature of 65°C. for rat consumption, weighed 450 gm. When ready for feeding, assays of the dried diets were made for their thiamine and riboflavin contents. We employed the method of Hennessy ('41) for our thiamine determinations and that of Conner and Straub ('41) for the riboflavin content. The results of these assays have been summarized into table 1.

Thirty-two weanling white rats, 22 days old, were selected from our breeding colony and arranged into four groups. The average weight of the animals was 33.7 gm. They were housed in metal cages provided with raised floors made of 3 mesh to the inch wire screen. Water was given ad libitum. A group of eight animals were provided for each of the four diets studied. Observations were continued for 18 weeks, the animals being weighed weekly. The average amount of food consumed each day was determined for the first 10 weeks. At the end of this period the blood of each animal was sampled by cardiac puncture, and the total numbers and volumes of erythrocytes, hemoglobin levels and reticulocyte percentages determined.

RESULTS

1. Body weight. The curves setting forth the changes in the weights of these animals, recorded at 2-week intervals, are shown in figure 1. No significant differences in average weights were detected at the end of the second and fourth weeks. The data observed at 6, 10, 14 and 18 weeks have been condensed, and the average weights with their standard errors presented in table 2. When differences between these mean

[1] These pure vitamins were courteously provided by Merck and Company.

body weights were computed at each of the respective time intervals and their standard errors determined, the animals feeding on diet A were found to be significantly heavier than those eating either diet B or diet C. But there were no statistically significant differences in the weights of animals eating diets B and C, even at the end of the period of observation,

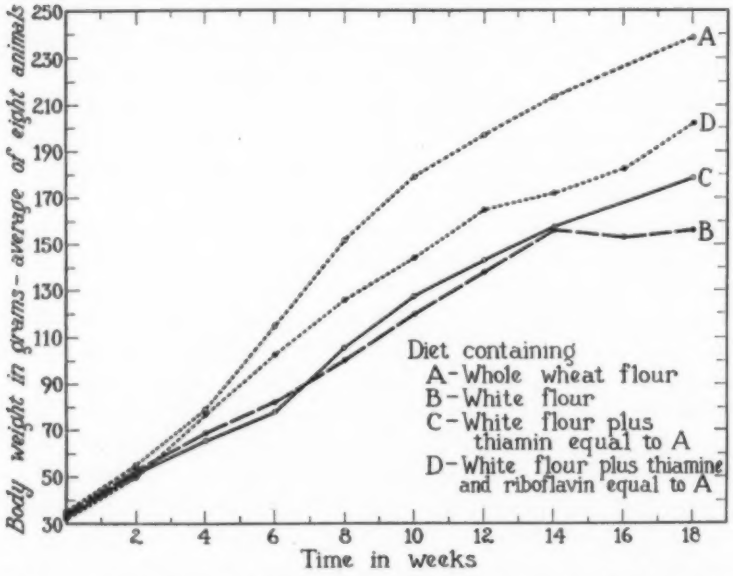

Fig. 1 Growth curves of four groups of rats fed the human diets in which the only variable was the flour from which the bread component was made.

18 weeks. Furthermore there were no real differences in the weights of animals eating diet A and those eating diet D, although the curves of their mean weights (fig. 1) show considerable disparity.

The data show that fortification of white flour with thiamine alone did not significantly alter the curve depicting growth. But the addition of riboflavin and thiamine induced weight increases which did significantly alter the growth curve. The

large error (12.8 gm.) of the mean weight of rats fed diet A and that of the mean weight of rats fed diet D (11.1 gm.), at 18 weeks, indicate the wide spread of the data, a fact which makes it unsafe to conclude that animals failed to grow as

TABLE 2

Data on body weights, food intake and blood.

CATEGORY OF INTEREST	DIET			
	A	B	C	D
Body weight (gm.) at:				
6 weeks (8) [1]	115.0 ± 5.0 [2]	82.5 ± 4.1	78.5 ± 3.3	103.2 ± 4.8
10 weeks (8)	179.0 ± 6.2	120.4 ± 5.6	127.1 ± 5.1	144.5 ± 4.5
14 weeks (8)	214.0 ± 8.5	156.8 ± 6.7	150.7 ± 6.4	172.4 ± 7.7
18 weeks (8)	242.0 ± 12.8	156.0 ± 1.7	179.5 ± 8.5	203.0 ± 11.1
Food intake (gm./rat/day) during:				
2nd week	7.0	5.5	6.5	7.5
4th week	7.0	5.0	7.5	8.0
6th week	9.5	5.0	6.5	7.5
8th week	8.0	4.0	7.0	8.5
10th week	9.5	3.5	6.0	10.0
Blood				
Erythrocytes in millions/cu.mm.	9.4 ± 0.21 [2] (8.40–10.20) [3]	7.94 ± 0.56 (5.85–9.85)	8.17 ± 0.32 (6.84–9.50)	9.45 ± 0.13 (8.90–9.90)
Erythrocyte volume in cu. microns	48.5 ± 1.1 (45.1–51.1)	44.1 ± 1.1 (38.8–50.0)	48.8 ± 1.5 (41.1–51.5)	49.3 ± 0.9 (47.2–53.9)
Hemoglobin in gm./100 cc.	12.8 ± 0.2 (10.8–12.8)	8.7 ± 0.8 (6.3–11.6)	9.5 ± 0.5 (7.9–11.6)	12.6 ± 0.2 (12.0–13.2)
Reticulocytes in %	3.2 ± 0.5 (1.2–4.9)	6.1 ± 0.7 (1.6–9.6)	3.1 ± 0.5 (0.8–5.2)	2.3 ± 0.4 (0.8–3.4)

[1] Figures in parentheses indicate number of animals.

[2] Standard error.

[3] Figures in parentheses indicate range of values.

well on a diet in which the bread component was made of white flour fortified with thiamine and riboflavin as on a similar ration in which the bread was made of whole wheat. However, white flour alone, as the bread source, was clearly inadequate to produce as satisfactory growth, and white flour fortified with thiamine alone was equally inadequate.

2. Food intake. The average food intake per rat per day was determined for the first 10 weeks of the experiment. The average amounts of food consumed in grams per day per rat at the end of the second, fourth, sixth, eighth and tenth week are presented in table 2. For the first few days of the test all rats ate like amounts (4.0 to 4.5 gm. per day). At the end of the second week, rats eating diets A and D increased their intake considerably over those eating diet B, while those receiving the added thiamine ate about 20% more.

At the end of the second week, the animals were getting the following amounts of thiamine daily on the respective diets: diet B, 4.7 μg.; diet C, 11.6 μg.; diet D, 12.4 μg.; and diet A, 12.9 μg. Richardson, Hogan, Long and Itschner ('41) provided in their diet B a daily allowance of 20 μg. of thiamine per rat, an amount which presumably resulted in optimal caloric intake. Our test rats, on that basis, were taking suboptimal amounts and those eating our diet B were getting abnormally low amounts. At the end of the tenth week (table 2) the consumption of diet B per diem per rat had dropped progressively to 3.5 gm., while intake of the diet of whole wheat flour or of white flour fortified with both thiamine and riboflavin had increased to 9.5 and 10.0 gm., respectively. At the end of the tenth week, rats eating diet A were taking about 17.5 μg. of thiamine daily; those eating diet B, about 3 μg.; those eating diet C, about 10.7 μg.; and those eating diet D, about 16.6 μg.

The riboflavin content of these diets was appreciably higher than the thiamine content (table 1). During the second week, the riboflavin intake per rat per day was as follows: diet A, 25.2 μg.; diet B, 13.7; diet C, 17.5; and diet D, 29.2. If the daily allowance of 20 μg. riboflavin per rat is optimal (Richardson and associates, '41), it is obvious that there was a lack of both riboflavin and thiamine in animals eating diets B and C.

3. Appearance of the animals. Characteristic cutaneous lesions, such as generalized dermatitis or acrodynia on paws, tail or ears, which develop in animals on synthetic diets when

some of the B fractions are withheld, never appeared in these animals. There was no bloody nasal discharge, nor encrustations on tips of ears or margins of toes; however, alopecia of a variable extent developed in rats which ate the basal ration B. Woolley ('40) learned that the antialopecia factor for mice is inositol (or its derivatives). Alopecia develops among riboflavin deficient rats but since our rats were taking this vitamin in considerable amounts it is not likely that riboflavin deficiency had caused the loss of hair. Degrees of alopecia, less marked than those seen in the B diet group, occurred in the C diet and D diet groups as well. Thus, the additions of thiamine and riboflavin to the basal diet, in the amounts indicated, did not completely inhibit the loss of hair. Alopecia, however, did not occur at all in rats eating the diet which included whole wheat bread.

4. Some observations on the blood at the end of the tenth week. As the experiment continued it became obvious that some of the animals eating diet B and some eating diet C were anemic, as judged by the color of their eyes. Accordingly, at the end of the tenth week, samples of blood were taken from the hearts of animals for a study of the total number of erythrocytes, their volumes, the percentage of reticulocytes and the hemoglobin levels.

Data assembled from these blood examinations are presented in table 2. The means of all determinations together with their standard errors and ranges observed are included. Although a considerable disparity appears between the mean erythrocyte counts of animals eating diets A and B, owing to the large error characteristic of the B diet data the difference is not statistically significant, being slightly less than three times its own error. Statistically significant differences of the mean volumes of erythrocytes, hemoglobin levels and reticulocyte percentages between animals eating diets A and B did exist and the conclusion that diet B had untoward effects on the erythrocytes would seem established. This was particularly true with respect to the hemoglobin levels and the sig-

nificantly elevated reticulocyte percentages. The data show that the anemia induced was of the hypochromic microcytic type.

The addition of the thiamine, while improving the erythrocyte count somewhat, did not significantly alter the hemoglobin level, it being statistically lower than the level for the A diet group. The addition of riboflavin and thiamine gave hematologic data entirely resembling those assembled from animals eating the whole wheat bread. Rats on diet D at the end of the tenth week were taking 39 μg. of riboflavin daily; while those eating diet C were taking but 16.2 μg. daily. Diet D rats were producing at this time 12.6 gm. of hemoglobin per 100 cc. of blood, while diet C rats produced but 9.5 gm. of hemoglobin. György, Robscheit-Robbins and Whipple ('38) showed that riboflavin increased hemoglobin production in the anemic dog.

Street, Cowgill and Zimmerman ('41) developed microcytic hypochromic anemia in dogs on a synthetic diet deficient in pyridoxine. The administration of this fraction to such anemic dogs caused a rapid increase of both hemoglobin concentration and erythrocyte counts. Similar results had been observed by Fouts, Helmer, Lepkovsky and Jukes ('38) among puppies fed a diet deficient in this B fraction. Later Fouts, Helmer and Lepkovsky ('40) showed that similar reactions occurred among adult dogs. Assay of our diet for its pyridoxine content was not made, so that we have no data with respect to the relation of this fraction to the microcytic hypochromic anemia which was induced.

The dyscrasia was not prevented by iron, for the administration of ferrous sulfate to a series of rats fed the basal ration did not protect against the anemia which developed in young rats. This observation is in keeping with statements previously made that iron was without effect on the anemia induced on synthetic diets deficient in B_6.

SUMMARY AND CONCLUSIONS

The purpose of this study was to observe the effects on growing white rats of feeding human diets in which the bread component was the only variable.

The addition of thiamine to the flour of diet B to an extent which doubled the thiamine intake for each gram of food consumed, did not induce any significant change of the growth curve. The addition of thiamine and riboflavin to the flour of the basal diet did increase the growth rate significantly. Such addition of thiamine and riboflavin to the basal diet induced weight increases which were statistically equal to those attained by rats eating the diet in which the bread was made of whole wheat flour.

The food intake per rat per day showed clearly the value of fortification of the flour with thiamine and riboflavin. The intake per rat per day of the diet fortified by these vitamins was equal to that of rats eating the diet of whole wheat flour.

The addition of thiamine to the flour did not increase the level of hemoglobin, but the addition of thiamine and riboflavin produced hemoglobin levels which were similar to those obtained on the whole wheat diet. These data confirm earlier observations that riboflavin increased hemoglobin production in the dog. The total erythrocyte counts, the cell volumes and reticulocyte percentages in rats eating diets A and D were statistically alike.

These data show that feeding to rats human diets, adequate with respect to proteins, carbohydrates, fats, vitamins A, C and D and essential minerals, but deficient in certain fractions of the B-complex, induced certain well-defined physical changes. These changes, such as body weight, gross appearance, and the hypochromic microcytic anemia, were improved or rectified by adding thiamine and riboflavin to the flour from which the bread component of the human diets was made.

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THE EFFECT OF CHLORIDE WITH HIGH AND LOW IODINE SUPPLY ON THE THYROID GLAND OF THE RAT

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In studies on experimental goiter, calcium chloride has frequently been used as a goitrogenic agent. However, calcium and not chlorine was the factor studied. Hibbard ('33) confirmed the goiter producing action of calcium chloride and carried the work further by studying sodium chloride and calcium lactate. His conclusion was that sodium chloride was as effective as calcium chloride in producing hyperplasia of the thyroid, and therefore that chloride was the goitrogenic agent. Remington ('37) concluded from experiments with a different low iodine diet that chloride was not goitrogenic. Although they arrived at opposite conclusions, the results are not wholly incompatible because different criteria of goiter were used by the two investigators. Hibbard's conclusions were based on microscopic structure, whereas Remington's were based on weight, per cent dry matter, and iodine concentration of the thyroid. Goiter means enlarged thyroid, hence, Remington's conclusion is probably more literally correct. However, Remington's figures show that sodium chloride caused a diminution of the iodine concentration and also per cent dry matter in the thyroid, results which indicate a probable change in the microscopic structure similar to that described by Hibbard.

In experiments designed to determine the goitrogenic effect of calcium, Sharpless, Sabol, Anthony and Argetsinger ('43)

found that under proper conditions calcium chloride produced thyroid enlargement, but that calcium carbonate did not. This is the report of further studies concerning the effect of chlorides on the thyroid.

EXPERIMENTAL

The methods used in this study were the same as those described previously (Sharpless, Pearsons and Prato, '39). In all studies of experimental goiter, one of the prerequisites is to lower the glandular iodine concentration. The iodine content is usually decreased but may remain within normal limits. A diet containing considerable iodine rather than one in which the iodine content is low might show more clearly the tendency of chloride to lower the glandular iodine concentration. For that reason two diets, one with a relatively low, and the other with a high content of iodine, were studied. The percentage composition of the basal diet was as follows: soy bean flour (unprocessed) 75.0, sucrose 15.5, dried brewers' yeast 3.0, butterfat 5.0, sodium chloride 1.0, calcium carbonate 0.5, and 4.0 μg. of iodine as potassium iodide per 100 gm. of diet. In the second diet the iodine supplement was increased to 50 μg. of iodine per 100 gm.

The chlorides incorporated in the diets were the sodium and ammonium salts, hydrochloric acid and a combination of calcium chloride with hydrochloric acid. Hydrochloric acid and ammonium chloride tend to produce acidosis, and the chloride of calcium chloride has approximately 75% of the acidosis producing effect of hydrochloric acid.

Blood iodine was determined in some of the rats when they were killed.

RESULTS

The results are recorded in table 1. The first four diets contain approximately 9.0 μg. of iodine per 100 gm., 4.0 μg. of which are obtained from potassium iodide.

Determinations of iodine in the glands of nine rats fed ammonium chloride were not obtained but in each of the other

chloride containing diets the iodine concentration was reduced between 30 and 50%. This was accomplished without a significant change in the fresh thyroid weight.

In the series of diets supplemented with 50 μg. of iodine per 100 gm., the average iodine concentration in the glands of rats receiving no chloride was 0.168% of the dry weight. Inclusion of 2.1% sodium chloride in this diet reduced the iodine concentration to 0.095%. In view of the difficulty in getting an accurate estimation of the iodine in glands containing less than .05% iodine, this reduction is considered more significant than the results with the low iodine diet.

TABLE 1

Effect of chloride on thyroid weight and iodine concentration.

BASAL DIET SUPPLEMENTS	NO. OF RATS	BODY WEIGHT		THYROID WEIGHT		IODINE	
		Start	Final	Ave. total	Per 100 gm. body wt.	Total	Dry basis
		gm.	*gm.*	*mg.*	*mg.*	*μg.*	*%*
Basal	91	65	140	23	17 ± .33	2.4	.041
2.1% NaCl	18	67	148	24	17 ± .46	1.2	.021
15 cc. HCl	25	65	155	29	19 ± .84	1.6	.025
2% NH_4Cl	9	87	144	24	17 ± .70	..	...
50 I_2 per 100 gm.	13	66	130	14	11 ± .67	5.3	.168
2.1% NaCl + I_2	14	66	120	15	13 ± .53	3.6	.095
$CaCl_2$ + I_2	14	70	145	14	10 ± .46	4.7	.123
HCl + $CaCl_2$ + I_2	14	68	133	12	10 ± .59	3.7	.111
HCl + I_2	11	64	130	14	11 ± .56	4.8	.136

The increase in thyroid weight from 11 mg. per 100 gm. of body weight for the controls to 13 for the rats fed an excess of sodium chloride was not mathematically significant. Supplements of hydrochloric acid alone or combined with calcium chloride resulted in considerable decrease in the iodine concentration but had no effect on the thyroid weight.

In the experiments with three of the diets, the iodine concentration in the blood, expressed as micrograms per 100 cc. and determined when the animals were killed, was as follows: basal, 3.7; basal plus 50 μg. of iodine, 10.5; basal plus 50 μg. of iodine and 2.1% sodium chloride, 4.1.

DISCUSSION

The results consistently show that the thyroid iodine concentration can be decreased by feeding an excess of chloride. This decrease is most clearly demonstrated when there is considerable iodine in the diet. The conclusion that chloride is not goitrogenic appears valid since chloride did not cause a significant increase in thyroid weight in any of the experiments.

An iodine deficiency is assumed to be present in the basal diet because addition of 50 μg. of iodine per 100 gm. reduces the thyroid weight. Furthermore, previous experiments (Sharpless, Pearsons and Prato, '39) have shown that the thyroid weight is greatly increased when an iodine supplement is omitted from the basal diet. It is the accepted theory that a work hypertrophy and, hence, thyroid enlargement occurs when there is insufficient iodine to produce the required amount of thyroxine. Regardless of iodine intake this theory is supported by the well-known fact that the remaining portion will become hypertrophied after a large part of the gland has been excised. If it is assumed that the thyroxine requirement is unchanged, it follows that the amount of hormone released by glands of chloride-fed animals must be regarded as unchanged. Since it was observed that sodium chloride reduced the blood iodine and all of the chloride-rich diets reduced the thyroid iodine concentration, there should be less iodine available for thyroxine formation, and therefore a compensatory enlargement of the gland would be expected. In experimental goiter studies where iodine intake is the variable factor, thyroid iodine concentrations below 0.1% of the dry weight are believed to bear an inverse relation to the fresh weight of the gland. There appears to be a paradox in the studies, in that a decrease in iodine intake will produce thyroid enlargement, yet a reduction of the iodine concentration in the animal has no effect on thyroid weight.

It has long been recognized in goiter studies that a relative iodine deficiency is the important predisposing factor, which implies that positive factors influence the utilization of iodine or increase the requirement for thyroxine. Unpublished

studies in this laboratory have demonstrated that sulfaguanidine inhibits the formation of thyroxine without appreciably changing the requirement for this hormone. Previous studies (Sharpless, Pearsons and Prato, '39) have shown that a positive factor in soybeans increases the iodine requirement, and calcium chloride when fed with vitamin D is goitrogenic (Sharpless, Sabol, Anthony and Argetsinger, '43). The studies reported here were designed to determine whether chloride could produce goiter. This question has been answered but in order to explain the negative results the effect of excess chloride and the consequent large volume of urine on excretion of positive goitrogenic factors should be investigated.

CONCLUSIONS

Feeding an excess of chloride decreases the iodine concentration of the thyroid in both normal and iodine deficient glands.

Despite the decreased iodine concentration in the glands, an excess of chloride did not significantly change the thyroid weight.

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THE BASAL METABOLISM OF NORMAL COLLEGE WOMEN

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INTRODUCTION

Studies on the basal metabolism of normal college women have been reported from time to time. The data of McKittrick ('36) on one hundred women at the University of Wyoming show a higher basal metabolism than those on subjects living nearer sea level. The figures given by Coons ('31) and Coons and Schiefelbusch ('32) on ninety-five women at Oklahoma Agricultural and Mechanical College are approximately 10% lower than those of McKittrick. Tilt ('30), reporting on fifty-two women at Florida State College for Women, gives figures slightly above those of Coons, whereas the figures of Tilt and Walters ('35) from a later study are lower than those of Coons.

PROCEDURE

Subjects. The subjects were 124 young women selected on the basis of health, age, and the altitude at which they had lived for the 6-month period previous to the determination of their basal metabolism. The weight of each subject was within the normal range of -10% to $+20\%$ of the average, according to the Baldwin-Wood, and the Wood tables.

The subjects ranged in age from 18 through 38 years with 105 of the 124 subjects being within the range of 18 to 22 years. Those from 23 to 38 years of age were placed in one group, since it has been shown that the metabolism does not change

appreciably during this period. The age range of 18 to 38 years was comparable with that of other similar studies. The age recorded was that of the subject at her nearest birthday. The subjects were selected from a region ranging in altitude from 2600 to 3800 feet. The altitude of the campus of Texas Technological College is 3215 feet.

The age groups, number of subjects and average height and weight as well as other data are recorded in table 3. The heights ranged from 153.1 to 179.8 cm.; the range of weights was from 45.9 to 70.9 kg. The range of surface area, as calculated from the DuBois height-weight formula, was 1.40 to 1.86 sq. m., with an average of 1.63.

Seven of the subjects showed a greater negative deviation in weight than 10% from the average for their age and height, three of these being in the oldest group (23 to 38 years). The maximum negative deviation accepted was 15.4% for L. A., a person of small skeletal build. Only five subjects were heavier than 15% and none were more than 20% above the accepted average for their height and age. The average deviation from normal was — 1.6%. All the subjects therefore were normal specimens, each being representative of her age.

Apparatus. A Collins' Benedict-Roth Recording Metabolism Apparatus was used to determine the oxygen consumption of the subjects.

Technique. The standard experimental conditions for the determination of basal metabolism were carefully observed.

To assure standard basal metabolism conditions on the morning of the determination, the subject was cautioned to take no food after the evening meal, to sleep at least 8 hours, to omit taking a bath, to forego smoking, and to avoid any unnecessary muscular exertion. Each subject was brought to the laboratory in a car, arriving at 7:30 or 8:30 A.M., approximately 13 to 15 hours after the last intake of food, and lay on a cot in a quiet room, comfortably warm, for 30 to 40 minutes before the determination.

At the end of the rest period, each subject's sublingual temperature was determined. If her temperature was not within the normal range, no determination was made. The pulse was counted several times before, as well as during, the determination. The procedure was explained to the subject, a precaution which helped in securing her cooperation.

If the subject showed signs of discomfort the determination was delayed until she was apparently relaxed. Care was taken to prevent her falling asleep during the determination. Two determinations of 8 to 10 minutes' duration were made on each morning with a few minutes' rest between. If the first 2 or 3 minutes of the determination were irregular, they were discarded when plotting the oxygen line. Determinations were made on at least two mornings and continued, if necessary, until the lowest figure obtained for oxygen consumption was checked within 5% by a result obtained on a different morning. The lowest figure and those within 5% were averaged to obtain the basal oxygen consumption. Since it is accepted that this method of indirect calorimetry is accurate only within 5%, the authors believe that an average of the lowest oxygen consumption and those figures within 5% of it represents the basal oxygen consumption more accurately than does the lowest figure alone.

From the basal oxygen consumption, the Calories per 24 hours, per hour and per square meter per hour were calculated. The basal metabolism of the subject was compared with the Aub-DuBois, Harris-Benedict, Dreyer, and Mayo standards and predictions.

RESULTS

The basal metabolism of the subjects was remarkably uniform, showing small variations between the individuals as well as between age groups. The metabolism, when expressed as Calories per 24 hours, is near the general average of 1300 Calories. The smallest range in the Calorie expenditure occurs when expressing the metabolism as Calories per square meter per hour. On this basis the highest Calorie expenditure

TABLE 1

Basal metabolism of subjects with deviations from standards.

AGE IN YEARS	NO. OF SUBJ.	CALORIES PER 24 HOURS		CALORIES PER HOUR				PER CENT DEVIATIONS FROM STANDARDS			
				Per sq. m.		Per kg.		Harris-Benedict	Dreyer	Aub-DuBois	Mayo
		Av.	Range	Av.	Range	Av.	Range				
18	18	1310	1128–1424	33.8	31.1–36.6	0.97	0.88–1.04	— 7.5	— 8.8	— 11.0	— 8.8
19	25	1323	1182–1482	33.5	30.2–37.9	0.94	0.81–1.05	— 7.7	— 8.8	— 11.9	— 8.7
20	25	1321	1161–1540	33.7	30.8–36.8	0.96	0.81–1.08	— 7.0	— 7.6	— 8.9	— 7.4
21	20	1316	1180–1535	33.6	30.3–36.6	0.96	0.79–1.10	— 6.8	— 7.0	— 9.1	— 7.2
22	17	1284	1146–1428	32.7	30.8–35.1	0.94	0.83–1.03	— 8.7	— 8.5	— 11.6	— 9.5
23–38	19	1268	1111–1479	32.8	29.5–37.1	0.93	0.81–1.08	— 7.6	— 7.0	— 10.7	— 8.5
18–38	124	1304	1111–1540	33.4	29.5–37.9	0.95	0.79–1.10	— 7.6	— 8.0	— 10.5	— 8.4

was 28% above the lowest, whereas, on the basis of Calories per 24 hours or per kilogram per hour, the highest value was 39% above the lowest.

A comparison of the basal metabolism of these subjects with the accepted standards and predictions shows that the metabolism of most of the subjects is within the range designated as "normal zone" in clinical practice, that is, + or — 15% of a standard.

The deviations from the Aub-DuBois standard range from + 2.7 to — 20.5%. Harris and Benedict's formula gives the closest agreement with the results of the actual determinations made on these subjects. The range of deviations is found to be from + 5.0 to — 19.0%. Seven deviations are greater than — 15% from the Harris-Benedict predictions in contrast with twenty-seven deviations which were greater than — 15% from the Aub-DuBois standard. Dreyer's formula gives results in close harmony with the Harris-Benedict predictions. The deviations from the Mayo standard range from + 3.1 to — 18.4%, with seven of the subjects showing a greater deviation than — 15%. The negative deviations from the Mayo standard are slightly greater than those from Harris-Benedict's formula.

The statistical analysis of the data is given is table 2. Whether the differences found in the Calorie expenditure of the various age groups are real or accidental seems to be an equal chance, with the exception of the difference between the 21- and 22-year age groups. When the average of Calories per square meter per hour for the 18- to 21-year-old group is compared statistically with the average for the 22- to 38-year group, the chances are greater that the difference is real but still not significant.

The statistical analysis shows that the subjects within an age group are homogeneous. When considering the variations between the age groups, the probable errors show that these also do not vary significantly.

TABLE 2

Statistical treatment of the means of calorie expenditures.

	AGE	NO. OF SUBJECTS	MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION	PROBABLE ERROR OF MEAN
	Year				%	
	18	18	1310	80.0	6.1	12.7
Calories	19	25	1323	97.8	7.4	13.2
per	20	25	1321	90.8	6.9	12.3
24 hours	21	20	1316	101.9	7.7	15.4
	22	17	1284	85.0	6.6	13.9
	23–38	19	1268	113.2	8.9	17.5
	18–38	124	1304	96.3	7.4	5.8
	18	18	33.8	1.41	4.2	0.22
Calories	19	25	33.5	1.96	5.9	0.26
per sq.m.	20	25	33.7	1.55	4.6	0.21
per hour	21	20	33.6	1.81	5.4	0.27
	22	17	32.7	1.52	4.6	0.24
	23–38	19	32.8	2.34	7.1	0.36
	18–38	124	33.4	1.81	5.4	0.11
	18	18	0.97	0.046	4.7	0.007
Calories	19	25	0.94	0.075	8.0	0.010
per kg.	20	25	0.96	0.063	6.6	0.009
per hour	21	20	0.96	0.069	7.2	0.010
	22	17	0.94	0.051	5.4	0.008
	23–38	19	0.93	0.078	8.4	0.012
	18–38	124	0.95	0.065	6.9	0.004

COMPARISON WITH OTHER STUDIES

When the metabolism is expressed as Calories per 24 hours, the size of the subject is not a factor for consideration and a comparison of the basal metabolism of different individuals on this basis is hardly worthwhile. When comparing the basal metabolism per square meter per hour of the different groups, the averages of the 18- and 19-year-old groups of McKittrick are higher than those of any other groups with the exception of the 18-year-olds of Blunt et al. ('26). The differences between the averages of the various studies are neither large nor consistent.

In comparing the deviations of the basal metabolism from standards, the deviations from Harris-Benedict and Aub-DuBois are used for comparison with those reported in the literature. The smallest negative deviations for each age group are those of McKittrick ('36). The largest are those of the studies of Coons ('31), Tilt ('30), and Tilt and Walters ('35). The deviations obtained in the study at Texas Technological College are usually intermediate between those of McKittrick and those of the other studies for the age groups except with respect to the 23- to 38-year-old group where those of Tilt ('30) are nearer McKittrick's.

The last section of table 3 compares the averages for the basal metabolism of the whole group of subjects with those of comparable studies. The subjects of the local study are the largest in stature. Their basal expenditure in Calories per square meter per hour does not vary consistently from other studies. The deviations of their metabolism from the Harris-Benedict and Aub-DuBois standards do not differ greatly from those of Tilt ('30), but are less negative than those of Coons ('31), Coons and Schiefelbusch ('32), and Tilt and Walters ('35), whereas all the negative deviations are greater than those of McKittrick ('36). Hetler ('32), reporting on eighty-five 19- to 37-year-old women students of the University of Illinois, found an average heat production of 1260 Calories per 24 hours. The negative deviation from the Harris-Benedict prediction was —7.1%, which is near that of this study. Rogers ('39), working with 110 college women, 17 to 27 years old from Connecticut State College, found average deviations of —1.9% from the Harris-Benedict and —2.3% from the Aub-DuBois standards. She attributes the small deviations to the excellent quality and quantity of the diets of the Connecticut women. Rogers' deviations are the only ones as low as those of McKittrick. McCord ('39), at the University of Indiana, found that the deviation of the basal metabolism of eight 18-year-old women was —7.5% from the Harris-Benedict and —8.1% from the modified Aub-DuBois

TABLE 3

Comparison of averages for age groups with others of comparable studies.

PLACE	NO. OF SUBJECTS	AGE	HT.	WT.	CALORIES PER 24 HR.	CALORIES PER SQ.M. PER HR.	DEVIATIONS FROM STANDARDS	
							Harris-Benedict	Aub-DuBois
		yr.	*cm.*	*kg.*				
Texas Tech.	18	18	164	56.6	1310	33.8	— 7.5	— 11.0
Okla. — Coons [1]	14	18	164	56.5	1265	32.8	— 10.6	— 13.7
Florida — Tilt [2]	6	18	157	54.4	1250	34.0	— 11.5	— 10.5
Wyo. — McKittrick	7	18	166	59.6	1654	36.0	— 1.8	— 3.5
Florida — Tilt [3]	6	18	165	56.1	1231	...	— 13.5	— 14.5 [4]
Ohio — McKay	15	18	164	59.9	1365	34.8	.....	.....
Wis. — Stark	50	17–18	...	...	1313	34.4	.....	.....
Ill. — Blunt	3	18	165	56.8	1406	36.7	.....	.....
Texas Tech.	25	19	165	58.7	1323	33.5	— 7.7	— 11.9
Okla. — Coons [1]	15	19	163	54.6	1235	32.5	— 11.1	— 14.4
Florida — Tilt [2]	7	19	160	55.5	1245	33.3	— 10.8	— 12.3
Wyo. — McKittrick	20	19	161	55.0	1340	36.0	— 3.4	— 3.8
Florida — Tilt [3]	6	19	166	58.1	1192	...	— 16.4	— 17.6 [4]
Wis. — Stark	47	19–20	...	...	1280	33.5	.....	.....
Texas Tech.	25	20	165	57.8	1321	33.7	— 7.0	— 8.9
Okla. — Coons [1]	14	20	163	55.1	1238	32.5	— 11.5	— 12.1
Florida — Tilt [2]	14	20	160	52.9	1240	33.6	— 8.6	— 9.3
Wyo. — McKittrick	24	20	163	56.7	1371	35.2	— 2.1	— 2.1
Florida — Tilt [3]	7	20	163	55.6	1198	...	— 14.0	— 14.3 [4]
Wis. — Stark	47	19–20	...	...	1280	33.5	.....	.....
Texas Tech.	20	21	166	57.3	1316	33.6	— 6.8	— 9.1
Okla. — Coons [1]	39	21–24	162	56.7	1248	32.6	— 10.5	— 11.9
Florida — Tilt [2]	12	21	158	55.1	1241	33.2	— 9.7	— 10.2
Wyo. — McKittrick	13	21	163	58.7	1379	35.8	— 2.7	— 3.0
Florida — Tilt [3]	5	21	165	57.3	1241	...	— 12.1	— 13.4
Texas Tech.	17	22	167	57.0	1284	32.7	— 8.7	— 11.6
Okla. — Coons [1]	39	21–24	162	56.7	1248	32.6	— 10.5	— 11.9
Florida — Tilt [2]	6	22	163	56.8	1237	32.5	— 9.8	— 12.2
Wyo. — McKittrick	11	22	162	56.0	1337	35.4	— 3.7	— 4.2
Texas Tech.	19	23–38	163	57.3	1268	32.8	— 7.6	— 10.7
Okla. — Coons [1]	13	25–39	164	57.1	1251	32.2	— 8.7	— 12.5
Florida — Tilt [2]	5	22–25	158	49.1	1227	34.6	— 6.1	— 6.7
Wyo. — McKittrick	19	23–26	163	57.8	1357	35.2	— 1.5	— 4.0
Florida — Tilt [3]	5	24–25	163	57.1	1236	...	— 10.9	— 12.1 [4]
Texas Tech.	124	18–38	165	57.5	1304	33.4	— 7.6	— 10.5
Okla. — Coons [1]	101	17–39	163	55.5	1245	32.6	— 10.1	— 13.2
Florida — Tilt [2]	52	17–25	159	54.5	1236	33.4	— 9.9	— 10.6
Wyo. — McKittrick	100	17–26	161	57.2	1368	35.6	— 2.5	— 3.2
Florida — Tilt [3]	30	17–25	164	56.4	1236	...	— 13.0	— 14.1 [4]
Okla. — Coons [5]	18	(College age)	162	51.9	1193	...	.....	— 12.0

[1] Study reported by Coons ('31).
[2] Study reported by Tilt ('30).
[3] Study reported by Tilt and Walters ('35).
[4] Modified DuBois Standard.
[5] Study reported by Coons and Schiefelbusch ('32).

standards. These deviations agree closely with those of the local study.

SUMMARY

The basal metabolism of 124 women ranging in age from 18 to 38 years was determined with a Benedict-Roth metabolism apparatus.

There was no significant variation between the age groups. The average basal metabolism of the subjects is 1304 Calories per 24 hours, 33.4 Calories per square meter per hour, or 0.95 Calories per kilogram per hour. The average deviations of the basal metabolism from the standards are —7.6% from Harris-Benedict, —8.0% from Dreyer, —10.5% from Aub-DuBois and —8.4% from Mayo.

The subjects of this study are larger in stature than those of any other group reported.

In comparing the results obtained on these subjects with those of comparable groups, the data do not differ consistently. The results of this study are usually intermediate between the highest basal metabolism reported (McKittrick, '36) and the lower ones (Coons, '31; Coons and Schiefelbusch, '32; Tilt, '30; Tilt and Walters, '35). The studies involving fewer subjects of a more limited age range are not statistically comparable. The differences cannot be attributed to altitude, however, since the data so far do not show any consistency. It is the opinion of the authors that any differences which may occur are the result of slight but significant differences in technique and interpretation of the data which various authors have made. Some of these may include: (1) whether the metabolism of a subject is determined on at least two different mornings before any of the figures are accepted as the basal metabolism; (2) the method of determining the oxygen line; (3) whether the lowest figure for oxygen consumption is used or some average of the figures; and (4) the care used in adjusting the subjects to the procedure. These and possibly other differences make it difficult to determine the significance of the variations found in the results of the various workers.

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INFLUENCE OF LECITHIN FEEDING ON FAT AND VITAMIN A ABSORPTION IN MAN [1]

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The effect on blood Ca, P and cholesterol in man as a result of feeding of large amounts of lecithin is being reported elsewhere. The innocuousness of large amounts (up to 20 gm. per dose and 70 gm. per day) of commercial lecithin was demonstrated in these studies. In contrast to purified lecithin preparations commercial lecithin has only recently become available at a price which facilitates the clinical administration of large amounts. The availability of large quantities renders possible a study of its effects upon intestinal absorption of fat and fat soluble vitamins (Reiser, '39). Improvement of intestinal fat absorption may find therapeutic application in the treatment of conditions associated with malabsorption of fat, like sprue, celiac disease, enteropathy in deficiency states, etc. (Adlersberg and Weingarten, '41).

EXPERIMENTAL

Fat absorption

For our clinical studies we adopted as a standard procedure the fat tolerance test recommended by Nissen ('33) and modified by Kann and Sobotka ('42). The test determines the total serum lipids at various intervals before and after ingestion of 1 gm. of butterfat (given as heavy cream) per

[1] This investigation was aided by a grant from the American Lecithin Corporation, Elmhurst, New York.

kilogram of body weight at 9 A.M., after a fast of at least 12 hours. The maximum rise of the total lipids in the blood occurs between the fourth and fifth hour after ingestion, depending on the position and activity of the subject; under conditions of rest in bed the maximum elevation is found in the fifth hour.

The total fasting serum lipid content was first studied in a control group comprising sixteen normal individuals and patients convalescing from various internal diseases not involving the gastrointestinal tract. A second group consisted of patients suffering from gastrointestinal disease, especially chronic diarrhea, in whom an alteration of intestinal absorption could be anticipated; there were five cases of sprue, three of jejuno-ileitis, and one each of jejunal ulcer, intestinal tuberculosis, and megalojejunem. The total lipid content in the serum of the control group averaged 495 $\pm$ 31 mg. (SD_m = SD of mean) per cent with wide fluctuations from 275 to 895 mg.; in the gastrointestinal group, the average was slightly lower, 408 $\pm$ 29 mg. per cent with a range from 165 to 550. Whether or not this moderate lowering of the total serum lipids is actually caused by impaired intestinal fat absorption cannot be decided.

Fat tolerance test in control cases and sprue

Fat tolerance tests were performed on afebrile controls, cases of active sprue, and cases of sprue on remission. In the controls there was an average of 59% (range 25–129) increase in blood lipids 4 hours after ingestion of the fat tolerance meal.

In the second group, consisting of five typical cases of sprue with steatorrhea, macrocytic anemia, hypocalcemia, etc., the total lipid content of the serum remained practically unchanged (average increase 3%, range 0–12) after ingestion of the fat tolerance meal. This illustrates conclusively the inability of patients with active sprue to absorb fat.

The third group consisted of three typical cases of sprue who were examined after a prolonged treatment with special diet, liver extract, and vitamins, in the hospital, i.e., during a

remission. The total serum lipids increased 53% (range 32–75) after ingestion of the fat tolerance meal, indicating satisfactory fat absorption. The fat tolerance test represents a gauge for the absorptive capacity of the intestinal tract which can be advantageously used for the follow-up of cases of sprue and possibly other types of disturbed fat absorption.

Effect of lecithin on fat tolerance test

After the behavior in the fat tolerance test was established for controls as well as for cases of sprue, the effect of addition of 10–15 gm. commercial lecithin was studied. The lecithin was spread on four to six cookies, which were substituted for the usual slice of toast. The figures in table 1 show that

TABLE 1

Effect of lecithin on fat tolerance test.

GROUP	CREAM-TOAST-TEA (WITHOUT LECITHIN)				CREAM-TOAST-TEA (WITH LECITHIN)		
	Case	Total lipids in serum		Increase	Total lipids in serum		Increase
		Fasting	After 4 hours		Fasting	After 4 hours	
		mg.%	mg.%	%	mg.%	mg.%	%
Controls	137	360	450	25	397	556	42
	139	554	840	51	491	886	90
	144	478	609	27	578	1039	79
	146	729	929	27	736	1132	54
	147	377	499	32	349	664	90
	Mean	500 ± 67	665 ± 94	32	510 ± 69	855 ± 109	71
Sprue	101	524	544	4	393	489	24
	136	302	300	—1	241	337	31
	149	568	590	4	610	774	26
	Mean	465 ± 82	478 ± 90	2	315 ± 219	533 ± 128	27

fat absorption is increased by the addition of lecithin in every control case, the average increase being raised from 32 to 71%. In three cases of active sprue, addition of lecithin changed the picture from absence of absorption of fat to moderate absorption averaging 27%. Blank experiments with 10 gm. of lecithin but without the cream meal showed practically no increase of the blood lipids.

Vitamin A absorption

Little is known of the fate of vitamin A in the body; its level in the blood increases with its intake, and a low level in the blood can be considered an index of vitamin A deficiency (Lewis et al., '41, '42). After establishing the vitamin A and carotene contents in the serum of controls and cases with gastrointestinal disease, vitamin A tolerance was tested by the method described elsewhere (Kann and Sobotka, '42). The test consists of the determination of the vitamin A content of the serum in the fasting individual, and at frequent intervals after the ingestion of a test dose of vitamin A. Various amounts (1.5–15 cc.) of percomorph oil (1 cc. = 60,000 I.U. vitamin A)[2] were used for this purpose, but most tests were done with 3 cc. of percomorph oil (180,000 I.U. vitamin A). The maximum rise of vitamin A in the blood is found 4 hours after ingestion of the vitamin A tolerance test meal and thus parallels fat absorption.

Vitamin A and carotene content in "fasting" blood

Fifty-three determinations were performed on thirty-three subjects. The data are summarized in table 2. Eighteen of them were convalescing from various internal diseases and were afebrile for at least 7–10 days; of the other fifteen cases, twelve were suffering from gastrointestinal disease, especially chronic diarrhea, and three from icterus due to cirrhosis of the liver, carcinoma of the pancreas and hepatitis. The vitamin A of the fasting serum in the control group averaged 79 I.U. per cent ± 7 (range 35–185), the carotene 88 ± 5 μg. per cent (range 50–170). In the gastrointestinal group the figures were markedly lower, the average vitamin A level being 38 ± 3 I.U. per cent (range 25–61), and the average carotene level, 45 ± 5 μg. per cent (range 10–102). The lowering of the vitamin A and carotene contents in gastrointestinal and

[2] This figure is the titer as given by the manufacturer. Determinations of the product by the Dann and Evelyn method give lower values when the conversion factor 1600 is used.

liver disease is striking and confirms the findings of other authors (Lasch, '38; Clausen and McCoord, '38; Ralli et al., '41; Haig and Patek, '42). It is indicative of vitamin A deficiency in these cases and is probably caused by both diminished food intake and impaired intestinal absorption.

TABLE 2

Vitamin A and carotene in serum of gastrointestinal cases, especially in chronic diarrhea and liver disease.

CASE NO.	DIAGNOSIS	VITAMIN A	CAROTENE	CASE NO.	DIAGNOSIS	VITAMIN A	CAROTENE
		I.U./ 100 ml.	*μg./ 100 ml.*			*I.U./ 100 ml.*	*μg./ 100 ml.*
101	Sprue	41	31	116	Jejuno-ileitis (3)	54	64
101	Sprue	19	63	109	Megalojejunum	26	30
104	Sprue (2)[1]	41	19	118	Cirrhosis of liver	38	55
106	Sprue (2)	26	10	119	Carcinoma of pancreas	25	50
102	Psychoneurosis, colitis (2)	27	39	121	Hepatitis	58	50
103	Gastric ulcer (2)	26	22	Mean: Fifteen cases (24)		38 ± 3	45 ± 5
105	Intestinal tuberculosis (2)	26	34	Range: Eighteen afebrile controls (29)		35–185	50–170
107	Jejuno-ileitis (3)	45	53	Mean: Eighteen afebrile controls (29)		79 ± 7	88 ± 5
111	Jejuno-ileitis (3)	61	89				
112	Jejuno-ileitis (3)	48	102				

[1] Figures in parentheses following the diagnosis indicate number of specimens where more than one specimen was analyzed. Results given in these cases are averages.

Vitamin A tolerance test in control cases and sprue

Table 3 presents the results of vitamin A tolerance tests. In two control cases 600,000 I.U. of vitamin A were given and caused an elevation of the vitamin A level in the blood of over 300%. In seven individuals who ingested 90,000–180,000 I.U. of vitamin A, the group average vitamin A content of the blood rose 41% in 4 hours. The carotene content of the serum remained unchanged. In two cases of sprue examined in the active stage the ingestion of 90,000 I.U. of vitamin A failed to cause a rise in the blood's content of this factor. Thus, the vitamin A tolerance test gives results that parallel

TABLE 3

Vitamin A tolerance test without and with addition of lecithin.

CASE NO.	VITAMIN A GIVEN	TOLERANCE TEST WITHOUT LECITHIN			TOLERANCE TEST WITH LECITHIN		
	I.U.	Fasting	4 hours after test	Increase in %	Fasting	4 hours after test	Increase in %
		I.U./100 ml.			*I.U./100 ml.*		
110	600,000	57	248	335	—	—	—
114	600,000	41	179	336	—	—	—
114	90,000	31	59	40	—	—	—
115	90,000	65	151	132	—	—	—
124	180,000	—	—	—	185	408	120
126	180,000	—	—	—	147	219	49
128	180,000	—	—	—	95	187	97
116	120,000	61	79	29	42	100	138
117	180,000	39	65	67	54	230	326
120	180,000	99	162	65	39	128	228
122	180,000	118	123	4	83	137	48
123	180,000	83	125	51	114	308	170
125	180,000	82	112	37	81	226	179
127	180,000	98	162	65	90	445	395
Average of last 7 cases $\pm$ SD_m		83 $\pm$ 10	114 $\pm$ 12	41 [1]	72 $\pm$ 10	225 $\pm$ 45	212 [1]
Average of carotene of all cases (μg.)		(83 $\pm$ 5)	(82 $\pm$ 5)	(—1)	(88 $\pm$ 12)	(106 $\pm$ 13)	(23)
Two cases of active sprue							
101	90,000	41	38	—7	—	—	—
104	90,000	40	30	—25	—	—	—
Effect of defatted lecithin							
132	180,000	—	—	—	112	334	198
133	180,000	—	—	—	65	182	180
134	180,000	—	—	—	80	265	231
					86	260	202 [1]

[1] The average increase is computed as the difference between the average fasting value and the average 4-hour value.

those of the fat tolerance test as well as demonstrate the inability of patients with active sprue to absorb fat soluble vitamins.

Effect of lecithin on vitamin A tolerance test

In the next series the effect of lecithin was studied. Vitamin A tolerance tests were performed in these cases within an

interval of a few days, one test being made without lecithin and one with the addition of 9–12 gm. of commercial lecithin. The lecithin was spread on cookies which were substituted for the slice of bread. In the tests with lecithin the elevation is markedly enhanced, the vitamin A content rising 212% in the seven cases that had shown an average rise of only 41% when lecithin was not given. There also occurs a moderate elevation of the carotene content in the blood which is not present when the test is performed without lecithin. Hence, the addition of lecithin apparently promotes the absorption of vitamin A in a way similar to fat absorption. However, a possible mobilization of vitamin A depots by lecithin has to be considered.

Commercial lecithin represents a natural product of soya bean phosphatids with an approximate content of 20% lecithin and 30% soya oil.[3] The high content of soya oil made it advisable to perform a series of examinations with a defatted product. The last groups in table 3 summarize three tests performed with 180,000 I.U. of vitamin A plus 10 gm. of defatted commercial lecithin. The average per cent increase of vitamin A in the serum is 202; thus, the increases observed with commercial lecithin cannot be attributed to soya oil. Yet, the question of whether the effects of the commercial lecithin are caused exclusively by its lecithin content is unanswered. The effect of cephalin and inositol remains to be studied.

It was finally necessary to study the effect on the vitamin A and carotene contents of the blood of lecithin per se without the addition of vitamin A. Table 4 summarizes four tests in which the vitamin A and carotene contents were examined in fasting specimens and 4 hours after the administration of 12 gm. of commercial lecithin. In three cases there was a moderate increase of 5, 18 and 24% in the vitamin A content, while in the fourth case there was a mild decrease of 15%. The average percentual increase of 8, if at all significant, may be due to mobilization of vitamin A in the depots, probably in

[3] The approximate composition is: lecithin 20%; cephalin 20%; oil 30%; carbohydrates 10%; inositols and allied compounds 15%; phytosterols 2%.

the liver, by the lecithin, which is known to affect the fat metabolism of the liver and the mobilization of fat; it may exert a similar effect on the vitamin A stores. Further studies on the effect of lecithin on vitamin A depots are under way.

TABLE 4

Effect of commercial lecithin (12 gm.) on vitamin A content of serum.

CASE	VITAMIN A IN SERUM I.U./100 ML.			CAROTENE IN SERUM µG./100 ML.	
	Fasting	After 4 hours	% increase	Fasting	After 4 hours
128	102	126	24	100	125
129	91	96	5	93	93
130	72	85	18	50	70
131	101	86	—15	60	67
Mean	92 ± 34	98 ± 8	8	76 ± 12	89 ± 13

SUMMARY

The fat and vitamin A tolerance tests were adopted for the study of intestinal absorption in cases of sprue. Active sprue is characterized by absence of the usual elevation of the total lipids or of the vitamin A content of the serum following the administration of the test dose of fat or vitamin A, respectively.

Addition of lecithin to the fat and to the vitamin A used in the respective fat and vitamin A tolerance tests enhances the elevation of the total lipids or the vitamin A content of the serum, respectively. This effect is probably due to increased absorption and, perhaps to a lesser extent, to mobilization of hepatic deposits.

We are indebted for technical help to Mrs. Judith Braude.

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THE VITAMIN B COMPLEX OF PEELED WHEAT BREAD[1]

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ONE FIGURE

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The quality of the protein and the digestibility of the three major food substances in the whole wheat bread made from a new type of flour have been previously studied in this laboratory (Murlin et al., '41; Sealock et al., '41). The flour which is produced by the new "flotation process of Earle" is known as "peeled wheat" flour and represents 98% of the original weight of the wheat berry. In its preparation only the very thin epidermis or "beewing" is removed, leaving in the flour the major portion of the bran as well as the endosperm. In view of the long-recognized high vitamin and mineral contents of wheat bran as contrasted to those of the endosperm, the necessity of evaluating the other nutritional factors of the bread made from the flour is obvious.

It, therefore, has been the purpose of this investigation to determine the content of various members of the vitamin B complex in the peeled wheat bread and also in the flour. In addition, some of the vitamins have been determined in bread made with high vitamin yeast instead of regular yeast. In these cases the two kinds of bread were made from the same lot of flour, so that the only difference was the type of yeast.

[1] The authors are indebted to the Continental Baking Company for a research grant to defray a portion of the cost of this investigation.

[2] The data reported in this paper were taken from a thesis submitted by A. H. Livermore in partial fulfillment of the requirements for the Master of Science degree.

EXPERIMENTAL

The bread used for these assays was baked by a local bakery from the peeled wheat flour as furnished to the bakery, the loaves being delivered to the laboratory on the day of baking. The majority of the samples were obtained over a period of 6 months with occasional loaves being obtained in the succeeding 5 months. In some instances the loaves were crumbled and dried at room temperature and then ground to a fine powder before removing assay aliquots. In other instances the fresh loaf was immediately sampled by the quartering method and assayed.

The vitamins determined were those which are available in the crystalline state, and thus comparisons were made with the pure synthetic standard.[3] The assay methods are ones commonly used in this laboratory and elsewhere for determinations of this type. Strict attention has been paid to the details stipulated in each description in order that the method be used without essential modification.

Thiamine or vitamin B_1 was determined by means of the rat-growth method, employing a basal diet having the following percentage composition: vitamin-free casein 18, cornstarch 51, autoclaved or sulfite-treated (Kline et al., '38) yeast 15, salts 4, lard 8, cod liver oil 2 and celluflour 2. For each sample assayed four groups of six rats each were used. Two groups received the standard thiamine and the other two groups the material to be assayed, so that the final value for each sample was obtained by comparison of two levels of unknown with two levels of standard. For purpose of comparison the more recent fermentation procedure of Schultz, Atkin and Frey ('37) was used. With this method the blank or non-thiamine fermentation value of the bread samples was taken into account by proper use of aliquots in which the thiamine was destroyed by sulfite (Schultz et al., '41).

Both riboflavin and pyridoxine were assayed by rat-growth procedures. In the case of the former the basal diet had

[3] The crystalline vitamins were very kindly supplied by the Medical Department of Merck and Co., Inc.

the following percentage composition: vitamin-free casein 18, cornstarch and wheat extract (Bourquin and Sherman, '31) 66, salts 4, hydrogenated cottonseed oil [4] 8, cod liver oil 2 and celluflour 2. For each sample twenty-four rats were used, the division being the same as in the case of the thiamine assays. For the pyridoxine assays the procedure of Dimick and Schreffler ('39) was followed with the exception that 2% celluflour was substituted for an equivalent amount of sucrose. With the second sample assayed thirty instead of twenty-four rats were used, in order that three instead of two levels of the unknown might be fed. For the determination of pantothenic acid, the microbiological method of Pennington, Snell and Williams ('40), employing the organism, Lactobacillus casei ε, was used. The basal medium was modified by the addition of asparagine as suggested by Strong et al. ('41). The amount of growth obtained was determined by titration of the lactic acid formed after 72 hours' incubation. The microbiological procedure of Williams, Stout, Mitchell and McMahan ('41), employing the organism Saccharomyces cerevisiae, G. M., was used to determine inositol. In this method the amount of growth is determined by turbidimetric means.[5]

Since considerable difficulty was encountered in evaluating the nicotinic acid content of the peeled wheat bread, five different procedures depending on three different principles were used. Three modifications of the aniline-cyanogen bromide colorimetric method of determination were used. The method as modified by Arnold, Schreffler and Lipsius ('41) gave high nicotinic acid values with bread, as did also the modification suggested by Friedemann and Barborka ('41) in which freshly precipitated zinc hydroxide is used as a decolorizing agent. The original Melnick and Field method as described by Melnick, Oser and Siegel ('41) gave results much more comparable with the biological assays. Two biological methods were used: (1) the dog-weight procedure of Waisman.

[4] Crisco.

[5] Acknowledgment is made to Dr. R. J. Williams for the folic acid concentrate required in the basal media.

Mickelsen, McKibbin and Elvehjem ('40), the bread being fed a total of twelve different times to three dogs, and (2) the microbiological method of Snell and Wright ('41) employed to confirm the chemical and dog-weight determinations. The organism Lactobacillus arabinosus 17-5 was utilized in this method, the principle of which is similar to that described for pantothenic acid.

RESULTS

In table 1 the results of the thiamine, riboflavin, pyridoxine, pantothenic acid and inositol assays are summarized. In this table are also included thiamine, pantothenic acid and inositol values for the flour from which the bread was made.

TABLE 1

Thiamine, riboflavin, pyridoxine, pantothenic acid and inositol values.

VITAMIN	PEELED WHEAT BREAD OR FLOUR ASSAYED	ASSAY METHOD	NUMBER OF BAKERY BATCHES	NUMBER OF SAMPLES	VITAMIN CONTENT OF FRESH BREAD OR FLOUR
					μg./gm.
Thiamine	Bread	Fermentation	10	36	2.73 (±0.27)
	Bread	Rat growth	2	6	3.20 (±0.36)
	Bread (high vitamin yeast)	Fermentation	1	7	4.27 (±0.19)
		Rat growth	1	4	5.00 (±0.88)
	Flour	Fermentation	1	3	5.83 (±0.58)
Riboflavin	Bread	Rat growth	2	4	2.45 (±0.17)
Pyridoxine	Bread	Rat growth	1	2	3.14 (±0.08)
Pantothenic acid	Bread	Microbiological	5	19	5.2 (±1.6)
	Bread (high vitamin yeast)	Microbiological	1	14	4.9 (±1.9)
	Flour	Microbiological	1	4	9.6 (±3.0)
Inositol	Bread	Microbiological	2	7	644.0 (±224.0)
	Bread (high vitamin yeast)	Microbiological	1	4	365.0 (±249.0)
	Flour	Microbiological	1	2	1105.0 .....

The nicotinic acid values are summarized in table 2, in which the rather wide discrepancy between values obtained by different methods is readily apparent. The first three, namely, 30, 40 and 55.4 μg. per gram for the peeled wheat bread, are obviously in better agreement than the other two.

TABLE 2

Nicotinic acid values.

ASSAY METHOD	PEELED WHEAT BREAD OR FLOUR ASSAYED	NUMBER OF BAKERY BATCHES	NUMBER OF SAMPLES	VITAMIN CONTENT OF FRESH BREAD OR FLOUR	RECOVERY OF ADDED NICOTINIC ACID
				μg./gm.	%
Microbiological	Bread	3	12	30.0 (±5.0)	104
	Bread (high vitamin yeast)	1	6	44.4 (±5.2)	...
	Flour	1	3	47.7 (±9.9)	...
Dog-weight	Bread	2	3	40.0 (±6.0)	...
Melnick et al.	Bread	7	12	55.4 (±7.3)	106
	Bread (high vitamin yeast)	1	3	80.5 (±5.8)	...
	Flour	1	2	96.1	...
Arnold et al.	Bread	4	12	117.0 (±13.0)	104
	Bread (high vitamin yeast)	2	2	121.0	...
	Flour	1	6	92.0 (±11.0)	118
Friedemann and Barborka	Bread	3	21	174.0 (±21.0)	81
	Bread (high vitamin yeast)	1	3	217.0 (±32.0)	119
	Flour	1	1	213.0	91

Further, they are derived from three different types of assay procedures and yet exhibit the same order of magnitude even though the per cent discrepancy is rather large. However, for comparison with other food products the best value is probably obtained by averaging the first two, in which case the peeled wheat bread made with ordinary baker's yeast will be found to contain 35 μg. of nicotinic acid per gram of fresh bread.

The most striking feature in tables 1 and 2 is the difference in the thiamine and nicotinic acid contents resulting from the use of the high vitamin yeast.[6] In these batches of bread made from the same lot of flour and differing only in the type of yeast used, the thiamine and nicotinic acid of the finished product were increased by 67 and 27%, respectively.

An attempt has been made to compare the vitamin concentration of the peeled wheat bread with other types of bread in order to further evaluate it as a source of the B-complex. This comparison is summarized in table 3. As

TABLE 3

Vitamin content of wheat breads in micrograms per gram of fresh bread.

VITAMIN	BREAD				
	Peeled wheat				
	Regular yeast	High vitamin yeast	Whole wheat	White	Enriched white [1]
Thiamine	2.96	4.64	2.4 [2]	0.6 [2]	2.4– 4.8
Riboflavin	2.45		1.2 [2]	0.5 [2]	1.3– 2.6
Nicotinic acid	35.0	44.4	23.8 [2]	8.5 [2]	22.1–44.2
Pantothenic acid	5.2	4.9	4.6	2.8	

[1] Amounts established by The Food and Nutrition Board of the National Research Council.

[2] Accumulated average values obtained from the more recent literature.

indicated, some of the values are the average values derived from accumulated single determinations reported in the more recent publications from numerous laboratories. They represent the concentrations used in this laboratory for various purposes of calculation. The results of our own determination of pantothenic acid in whole wheat and white bread are also included.

The table shows that the new peeled wheat bread not only furnishes the vitamin concentration which might be expected

[6] High vitamin yeast, made by the Fleischmann Laboratories, contained the following per pound of the bakers' yeast in the moist state: iron 9.1 mg., nicotinic acid 82 to 103 mg., thiamine approximately 90 mg., riboflavin 11.2 mg. It was used in the amount of 3.5 pounds per 100 pounds of the flour.

from the inclusion of more of the whole grain in the flour, but also that it furnishes amounts of the three more important vitamins equal to the upper level established for the enrichment of white bread.

Further confirmation of the vitamin B-complex content of the peeled wheat bread was obtained by the type of experiment illustrated in figure 1. The percentage composition of the basal diet which was fed ad libitum was vitamin-free casein 18,

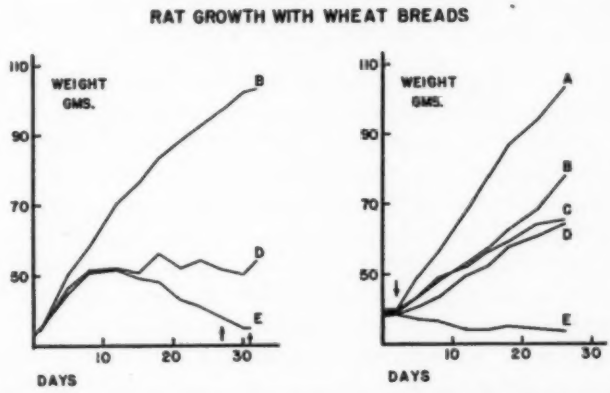

Fig. 1 Each curve represents the average growth of two animals maintained on a B complex-free basal diet, each animal receiving 3.0 gm. of the dried bread daily or other supplement as indicated. The crystalline vitamins included 50 μg. of thiamine, 50 of riboflavin, 40 of pyridoxine, 100 of calcium pantothenate and 1 mg. of inositol/day. A, crystalline vitamins; B, peeled wheat bread; C, whole wheat bread; D, white bread; E, no supplement. The arrow pointing downward indicates the beginning of supplementation, while each of those pointing upward indicates the death of one animal.

sucrose 66, salts 4, hydrogenated cottonseed oil [7] 8, cod liver oil 2 and celluflour 2. The white rats used for these experiments received in addition to the basal diet either 3 gm. daily of the dried breads, the crystalline vitamins or no supplement as indicated. Those represented in the graph to the left were depleted of their vitamin stores by feeding only the basal diet for 7 days before beginning supplementation.

[7] See footnote 4, page 267.

For the sake of brevity the curves shown represent the average weight changes of two animals in each case.

Since pantothenic acid appears to be the primary antigray hair factor for our colony of mixed black and piebald rats, no attempt was made to obtain a quantitative evaluation of the pigmentation factor as such. However, a qualitative experiment showed that the administration of between 2.5 and 5.0 gm. of the dried bread daily for 4 weeks resulted in a return of pigmentation comparable to that obtained with the administration of 25 μg. of calcium pantothenate daily.

Discussion of assay results of this type should include some reference to a point plainly evident to the majority of investigators. The assay methods in common use in this laboratory yield excellent and precise results when one assays a simple extract or solution of the vitamins. Difficulties which are encountered with the more complex materials are due primarily to the extraction procedure. Essentially, the difficulties are incompleteness of extraction, inability to break apart vitamin complexes, destruction of interfering substances and even the production of non-vitamin chromogens as in the chemical method for nicotinic acid. It is these which render inadequate the usual procedure of evaluating an analytical method, namely, the determination of the recovery of added substance under test. It is evident from the recovery values reported in table 2, that one may be misled. In other words, it may be concluded that recovery values indicate primarily that the final analytical procedure is operating satisfactorily and that marked destruction is not occurring in the process. However, little information is furnished concerning the other difficulties enumerated, particularly in regard to the completeness of extraction. The conclusion has been reached in this laboratory after several years of vitamin assays that the question of satisfactory extraction procedures represents the greatest difficulty in determining vitamin concentrations. Consequently, this problem is under investigation at present but further discussion must be reserved for the future.

SUMMARY

1. By means of standard assay procedures the content of various members of the vitamin B complex in bread made from peeled wheat flour has been determined.

2. The values obtained in micrograms per gram of fresh bread are thiamine 3.0, riboflavin 2.5, nicotinic acid 35, pantothenic acid 5.2, pyridoxine 3.1 and inositol 644.

3. The advantages of using a high vitamin yeast in baking the bread are evident in values for thiamine of 4.6, and for nicotinic acid of 44 as contrasted to the above values for bread made with regular yeast.

4. The peeled wheat flour was found to contain the following values in micrograms per gram: thiamine 5.8, nicotinic acid 72, pantothenic acid 9.6, and inositol 1100.

5. Confirmation of the assay values obtained is evident in the ability of the bread to supplement a vitamin B complex-free basal diet for the growth of white rats. In addition, its consumption results in the curing of gray hair in black rats in approximate proportion to its content of pantothenic acid.

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THE NICOTINIC ACID CONTENT OF COMMON FRUITS AND VEGETABLES AS PREPARED FOR HUMAN CONSUMPTION [1]

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Except for the summary prepared by Bacharach ('41) and a recent report by Teply, Strong and Elvehjem ('42), little attention has been given to the determination of the nicotinic acid content of vegetables and fruits. Since a daily allowance of nicotinic acid has been recommended by the Committee on Food and Nutrition of the National Research Council, it seemed especially important that information be gained of the quantity of this substance in vegetables and fruits in common use. Therefore, a rapid exploratory survey of a number of these foods was undertaken. It is realized that later detailed studies of each product found to be an important source of nicotinic acid will be necessary in order to determine what influence various factors, such as soil condition, variety, and storage conditions, may have upon the content of this substance.

The present report is concerned with the nicotinic acid content of raw vegetables and fruits and, if ordinarily cooked for the table, of the same foods immediately after being cooked. Data are also presented for certain dried legumes, canned foods and canned fruit juices.

[1] Journal series paper of the New Jersey Agricultural Experiment Station, Rutgers University, Department of Agricultural Biochemistry.

This work was done as part of the National Cooperative Experiment Station Project on Conservation of the Nutritive Value of Foods.

EXPERIMENTAL

Sampling and preparation for analyses. The foods were purchased in the open market without any reference to geographical origin, storage conditions or treatment following harvest. For analysis, 10 gm. were selected from a larger quantity — usually 200 gm. of the raw or cooked food — and chopped into fine pieces. The 10 gm. of fresh, or 3 gm. of dried vegetables or fruits were suspended in 200 cc. of water, autoclaved at 15 pounds for 30 minutes and made to volume, usually 200 cc. This suspension was placed in an electric blender for 5 minutes or until a fine, even dispersion was obtained. Any suspended material was included with the liquid in the test portions.

Cooking methods. The foods were cooked by a housewife according to methods suggested by the Home Economics Extension Service of the New Jersey Agricultural Experiment Station. The food was placed in a minimum quantity of boiling water and cooked in the minimum time necessary to make it palatable. Covered aluminum pans were used. Salt was added as seasoning. None of the foods was fried or baked.

Assay method. The microbiological method of Snell and Wright ('41) was employed essentially as described.

RESULTS AND DISCUSSION

Individual results rather than averages have been reported in the tables to emphasize the variations in nicotinic acid content which occur among samples of the same food obtained from the market at different times. Purchases of the same food were made at intervals of several weeks and, in a few cases, of several months.

For the most part, fresh vegetables which had been shipped from distant points were purchased during the winter and early spring. The nicotinic acid content of vegetables in the raw state (table 1) varied somewhat among samples of the same food. This may be due to such factors as variety, soil condition, or length of the storage or holding period. As might

TABLE 1

Nicotinic acid content of raw and cooked fresh vegetables.

FOOD	NICOTINIC ACID IN RAW FOOD	COOKED SOLIDS FROM 100 GM. OF RAW FOOD	NICOTINIC ACID IN SOLID PORTION OF COOKED FOOD		COOKING WATER REMAINING	NICOTINIC ACID IN COOKING WATER		LOSS DUE TO COOKING
	µg./100 gm.	*gm.*	*µg./100 gm.*	*%* [1]	*cc.*	*µg./cc.*	*%*	*%*
Roots and tubers								
Potatoes, sweet, yellow, peeled	527	105.0	473	94.4	11.0	1.6	3.3	2.3
	790	100.5	736	93.6	0.0	...	...	6.4
	627	101.5	510	81.5	2.5	9.3	3.7	14.8
Potatoes, white, peeled	364	100.0	280	77.0	50.0	1.4	19.4	3.6
	427	101.5	364	86.6	4.0	2.1	2.0	11.4
	555	101.0	510	92.7	7.5	3.0	4.1	3.2
Carrots, scraped	327	97.5	275	82.0	10.5	0.2	6.4	11.6
	345	90.0	275	71.7	25.0	1.7	12.3	16.0
	200	93.5	182	85.2	5.0	2.0	5.0	9.8
Beets, peeled	127	88.0	91	63.0	50.0	0.4	15.6	21.4
	164	87.0	127	67.5	55.0	0.9	30.2	2.3
	227	92.5	164	66.8	37.0	1.4	22.8	10.4
Legumes								
Peas	1480	100.0	973	65.5	90.0	5.4	32.7	1.8
	1380	113.0	927	76.0	20.5	14.5	21.6	2.4
	1820	102.0	1336	75.0	22.5	11.9	14.7	10.3
Beans, green	352	102.0	182	52.8	66.0	2.2	41.2	6.0
	355	113.0	264	84.0	5.5	6.8	10.6	5.4
	345	104.0	280	84.3	6.5	4.3	8.1	7.6
Beans, lima	1073	95.0	1000	88.5	0.0	...	...	11.5
	845	102.0	618	74.7	5.0	8.6	5.1	20.2
	845	105.0	720	89.5	27.5	4.1	13.3	+2.8
Flowers								
Broccoli	835	100.0	773	92.5	0.0	...	...	7.5
	1050	110.0	573	60.0	25.0	5.4	12.9	27.1
	790	102.0	455	58.7	21.0	2.3	6.1	35.2
Cauliflower	663	96.5	510	74.3	21.5	5.4	17.5	8.2
	600	102.0	410	69.8	21.5	3.8	13.6	16.6
	482	97.5	455	92.0	0.0	...	...	8.0
Leafy vegetables								
Beet greens	290	63.0	355	77.0	6.0	5.4	11.2	11.8
	327	75.0	364	83.5	0.0	...	...	16.5
Cabbage	118	84.5	109	78.0	0.0	...	...	22.0
	264	96.0	218	79.3	0.0	...	...	20.7
	264	92.0	200	69.7	6.0	1.8	4.1	26.2
Spinach	782	82.5	600	63.3	0.0	...	...	36.7
	720	85.0	627	74.0	8.5	4.7	5.6	20.4
	700	82.0	636	74.5	0.0	...	...	25.5
Miscellaneous								
Asparagus	1200	96.5	1100	88.4	5.5	9.1	4.2	7.4
	1155	99.0	1073	91.9	0.0	...	...	8.1
	1100	93.5	955	81.2	0.0	...	...	18.8

[1] This percentage was calculated from the quantity of nicotinic acid in the cooked solid portion from 100 gm. of raw food.

be expected the percentage of nicotinic acid in the raw food which was found to have been extracted by the cooking water was usually greater, the larger the volume of water. It varied from 2 to 41%.

The loss due to cooking, that is, the difference between the nicotinic acid content of the raw food and that of the cooked food and cooking water, varied with the type of food, and frequently among samples of the same food. For roots and tubers the average loss was 9% and for legumes 8%, whereas for the flowery plants it was 17% and for leafy plants 22%. This difference may be related to the surface of plant tissue exposed in proportion to volume, the flowery and leafy plants presenting a greater surface. Nicotinic acid is stable to oxidation and the temperature of ordinary cooking, so it is difficult to account for the loss observed. In keeping with household practice, cooking utensils were not rinsed when the cooked food was removed. In a few trials additional nicotinic acid was obtained by quantitative rinsing, but the total recovery was less than 100%.

While the present study was in progress, certain of the foods under examination were reported by Teply, Strong and Elvehjem ('42). Their results are in good agreement with those of this laboratory for green beans, dried lima beans, spinach, cabbage, cauliflower, orange juice and grapefruit juice. For the roots and tubers, sweet potatoes, beets, carrots and white potatoes, the values reported by Teply, Strong and Elvehjem ('42) are two to three times those found in our laboratory. This difference may be due, for example, to a difference in varieties or in length of storage prior to analysis.

The nicotinic acid contents of vegetables usually eaten in the raw state, of fresh fruits and of canned fruit juices are listed in table 2. Avocados, bananas, tomatoes, tomato juice and prune juice are the best sources of nicotinic acid among these foods.

Dried legumes were soaked in water overnight, the soaking water discarded and the legumes cooked in fresh water, a common household practice. In table 3 are shown the values

for dried legumes prepared for human consumption. The average loss of nicotinic acid, if the soaking water is discarded was 7%. Loss due to cooking averaged 13%, a value somewhat higher than the 8% observed for fresh legumes and due probably to the longer cooking time. Although lima

TABLE 2

Nicotinic acid content of vegetables usually consumed in the raw state, fresh fruits, and canned fruit juices.

VEGETABLES		FRUITS		CANNED JUICES	
Food	Nicotinic acid	Food	Nicotinic acid	Food	Nicotinic acid
	μg./100 gm.		*μg./100 gm.*		*μg./100 gm.*
Celery	264	Apples	91	Apricot	136
	190				
	182	Avocado	927	Grapefruit	154
			1020		154
Cucumbers	136		973		136
	109				
	168	Bananas	573	Orange	154
			464		164
Escarole	373		600		145
Green peppers	264				
	300	Cherries	127	Papaya	91
	237		145		
				Peach	300
Lettuce, head	100	Lemons	190		
	127		154	Pear	91
	200				
		Limes	275	Plum	118
Onions	182		227		
				Pineapple	136
Radishes	154	Oranges	218		
	136		173	Prune	427
	164		209		390
					455
Tomatoes	410	Strawberries	227		
	364		264	Tomato	510
	280				555
					564

beans and peas contain more nicotinic acid in the dry form than in the fresh state, the potency of the cooked solid portion of the latter is considerably greater than that of the cooked dried products, due possibly to the difference in maturity at the time of harvest.

TABLE 3

Nicotinic acid content of dried legumes before and after cooking.

FOOD	DRY STATE	SOAKING WATER FROM 100 GM. DRIED LEGUME	NICOTINIC ACID IN SOAKING WATER		COOKED SOLIDS FROM 100 GM. DRIED LEGUME	NICOTINIC ACID IN COOKING SOLIDS		COOKING WATER REMAINING	NICOTINIC ACID IN COOKING WATER		LOSS DUE TO COOKING [1]
	γ/100 gm.	*cc.*	*γ/cc.*	%	*gm.*	*γ/100 gm.*	% [1]	*cc.*	*γ/cc.*	%	%
Beans, black	1573	125	0.5	4.0	235	500	74.6	20	3.4	4.3	17.1
turtle	1325	80	1.0	6.0	245	455	84.1	0	..	..	9.9
Beans, kidney	1909	95	0.5	2.5	223	655	76.5	0	..	..	21.0
	1709	81	2.7	12.8	254	500	74.2	0	..	..	13.0
	1709	88	0.5	2.6	262	563	86.3	0	..	..	11.1
Beans, lima	1518	95	0.9	5.6	257	510	86.3	0	..	..	8.1
	1309	54	0.5	2.1	269	400	82.2	0	..	..	15.7
	1273	80	0.5	3.1	267	418	87.6	0	..	..	9.3
Beans, navy	1854	85	1.4	6.4	203	720	78.8	0	..	..	14.8
	1490	100	1.2	8.0	256	455	78.2	0	..	..	13.8
	1663	95	0.5	2.9	258	573	88.9	0	..	..	8.2
Beans, yellow	1800	150	1.4	11.7	250	464	64.4	55	5.1	15.6	8.3
eye	1490	90	2.7	16.3	213	525	75.0	0	..	..	8.7
Peas, split	2536	105	4.8	19.9	238	754	70.8	0	..	..	9.3
	2782	75	3.2	8.6	264	800	75.8	0	..	..	15.6
	2164	35	3.2	5.2	279	600	77.3	0	..	..	17.5

[1] Based on the nicotinic acid content of the dried legumes.

TABLE 4

Nicotinic acid content of canned vegetables before and after heating prior to serving.

FOOD	BEFORE HEATING							AFTER HEATING			Loss due to heating
	Weight of solid portion	Nicotinic acid in solid portion		Volume of liquid	Nicotinic acid in liquid portion			Total nicotinic acid			
								In solid portion	In liquid portion	Total contents	
	gm.	*µg./100 gm.*	*µg. total*	*cc.*	*µg./100 cc.*	*µg. total*	%	*µg.*	*µg.*	*µg.*	%
Beans, green	338	200	675	230	164	337	33	527	418	945	6.7
	337	163	555	245	182	445	45	534	418	952	4.8
	350	227	795	225	264	590	43	732	555	1286	7.2
Beans, lima	402	327	1318	200	336	672	34	1354	582	1936	2.8
	409	436	1786	185	290	536	23	1473	795	2268	2.4
	395	280	1114	204	245	507	31	1133	563	1697	+4.6
Peas	402	582	2345	185	636	1182	34	2273	1150	3423	3.1
	383	509	1945	200	555	1110	36	1810	973	2782	8.9
	400	763	3054	190	1000	1910	39	2982	1690	4673	5.9

In canned green beans, lima beans and peas (table 4), 30 to 40% of the nicotinic acid content was found in the liquid portion, an observation which emphasizes the importance of using the liquid portion of canned vegetables. The ratio of nicotinic acid in the liquid portion to that in the solid was essentially the same as the ratio of the liquid to the solid contents. Compared with the distribution obtained when fresh vegetables were cooked, the liquor in the can contained a much higher proportion of the vitamin than did the cooking water. This difference is undoubtedly due to the greater length of time the canned foods were in contact with the liquor. When the canned foods were brought just to the boiling point in

TABLE 5

Classification of fresh vegetables, dried legumes, fresh fruits and canned fruit juices, ready for human consumption, according to nicotinic acid content per 100 gm. of edible portion.

RANGE OF AMOUNT	VEGETABLES			LEGUMES	FRUITS	
	Raw	Cooked, fresh	Canned	Cooked dried	Fresh	Juices canned
100–500 μg./ 100 gm.	Celery Cucumbers Escarole Lettuce, head Onions Peppers, green Radishes Tomatoes	Beans, green Beets Beet greens Cabbage Carrots Cauliflower Potatoes, white	Beans, green [1] Beans, lima [1]	Beans, lima Beans, yellow eye	Apples Cherries Lemons Limes Oranges Straw-berries	Apricot Grapefruit Orange Papaya Peach Pear Pineapple Plum Prune
500–1000 μg./ 100 gm.		Beans, lima Broccoli Potatoes, sweet, yellow Spinach	Peas [1]	Beans, black turtle Beans, kidney Beans, navy Peas, split	Avocado Banana	Tomato
1000–1500 μg./ 100 gm.		Asparagus Peas				

[1] Per 100 gm. of solid and liquid contents.

a covered pan, as though in preparation for serving, the loss of nicotinic acid ranged from 2 to 10%.

In table 5, the foods examined have been placed in three groups according to whether the nicotinic acid content is between 1000 and 1500 μg., 500 and 1000 μg. or 100 and 500 μg. per 100 gm. This classification is suggested as a guide for use in the selection of foods until more extensive data have been obtained. The values observed for fruits and vegetables are much lower than 4000 to 5000 μg. per 100 gm. reported for muscle meats by Waisman and Elvehjem ('41). However, because the consumption of vegetables is usually larger than that of meat, many of the vegetables will contribute significant amounts of nicotinic acid to the dietary.

SUMMARY

By means of the microbiological method nicotinic acid was determined in thirteen fresh vegetables, in both the raw and the cooked state, eight fresh raw vegetables, six cooked dried legumes, three canned vegetables, seven fresh fruits and ten canned fruit juices. The nicotinic acid content was highest for fresh peas, asparagus and avocado pears and lowest for certain of the fruits and fruit juices. Seeds of legumes, both fresh and dried, gave fairly high values, while the values for root crops and blanched leaves were low. Considerable variation was found among different samples of the same crop. During cooking, there was a loss of the factor which averaged as follows: fresh legumes, 8%; roots and tubers, 9%; flowery plants, 17%; and leafy plants, 22%. In addition, the cooking water contained from 2 to 41% (average 12%) of the total nicotinic acid. The liquid associated with the canned vegetables contained from 30 to 40% of the total nicotinic acid.

ADDENDUM

Since the completion of this work an extensive review entitled "Nicotinic Acid in Foods" by R. W. McVicar and G. H. Berryman was published in The Journal of Nutrition, vol. 24, p. 235 ('42).

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NUTRITIVE ADEQUACY OF CERTAIN LOW-COST FOOD MIXTURES [1]

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This investigation represents an effort to find a combination of cheap foods that might be the basis for simple but adequate menus for very low-income groups in urban areas. These groups have been somewhat neglected in the present nutrition program. Limitation of income makes it impossible for most of the families concerned to follow the rules that have been set up relative to the kinds and amounts of food necessary for dietary adequacy, and many of the suggestions helpful in rural and suburban areas are impractical when all food must be bought at the neighborhood grocery store. Four different combinations of cheap foods, for each of which simple menus were devised, have been suggested [2] and calculated for adequacy. Calculations for three of these were on the basis of the requirements of a sedentary woman for 1 week, while one was on the basis of the requirements of a moderately active man for the same period of time.[3] In order to furnish proof of the adequacy of these food mixtures each of them was assayed for four vitamins and three minerals as follows: thiamine, niacin, pantothenic acid, riboflavin, calcium, phos-

[1] Supported in part by a grant from the Texas State Board of Health.

[2] These suggestions and calculations were made by members of a special committee of the State Nutrition Committee of Texas and published in the bulletin Better Meals for Less Money, issued by the Homemaking Division, State Board for Vocational Education.

[3] Recommended Daily Allowances for Specific Nutrients, Committee on Food and Nutrition, National Research Council.

phorus and iron. For comparison, food mixtures representative of both the minimum- and the moderate-cost diets of Carpenter and Stiebeling ('36) were similarly assayed, as was a stock diet that had been used successfully over a period of many years in feeding a rat colony. The last three mixtures are referred to as control diets, while the four proposed low-cost mixtures are referred to as test diets. In addition to assaying the food mixtures, each was fed to a group of young male albino rats and the resulting growth and food intake noted. The adequacy of a food mixture is often judged by the extent to which it promotes "normal" growth in experimental animals. There is no proof that a diet adequate for a growing rat will also be adequate for an adult man or vice versa, but differences in growth of animals resulting from different diets are generally considered to indicate differences in nutritive value.

VITAMIN AND MINERAL CONTENTS OF TEST AND CONTROL DIETS

Test diet 1 represents a very limited food selection. It was calculated for adequacy on the basis of a 3,000 calorie intake. Test diets 2 and 3 also represent limited food selection; the chief difference between them is that diet 2 contains no meat. Test diet 4 represents a more varied food selection and permits more appetizing menus than the other test diets. Diets 2, 3, and 4 were calculated as adequate on the basis of a 2100–2500 calorie intake. Cost of the diets varied from 8 to 12 cents per day per person in the fall of 1940. Market orders for all diets used are given in table 1 and are for 7 days for one adult, except test diet 4 which is for 6 days only.

In preparing the various food mixtures it was thought desirable to use ordinary cooking procedures so that the mixtures would be identical with the food as actually consumed, but at the same time it was necessary that the mixtures be suitable for rat feeding and for analysis. The general methods used are indicated in footnote 2 of table 1.

TABLE 1

Market orders for control and test diets.

FOODS	CONTROL DIETS			TEST DIETS			
	I[1]	II[2]	III[2]	1[2]	2[2]	3[2]	4[2]
	lb.	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*
Milk:							
Powder	1.93		.25				
Evaporated				1.88	1.81	1.81	
Skim							11.02
Whole		3.75	3.00				
Cheese			.25				
Vegetables and Fruits:							
Potatoes, white			1.75				1.72
Potatoes, sweet		1.50	1.50	3.00	1.00	2.00	.62
Tomatoes		1.00	2.00	.67	1.20	.63	.73
Grapefruit							.20
Oranges			.25				
Cabbage		.75	1.00		1.30		.58
Beet tops							.45
Turnip greens		1.00	1.00	1.00	1.00	1.00	
Carrots		1.00	1.00				.44
Beets			.50				.35
Turnips		.75	.75	1.00		1.00	
Onions			.75	1.00	1.00	1.00	.17
Dried beans or peas		.75	1.00	1.00	1.13	1.00	.22
Peanut butter			.12				
Peanuts, shelled					1.00		
Apples, dried			.25				.12
Apricots, dried		.50					
Peaches, dried			.25				.08
Prunes, dried			.25				
Raisins							.10
Eggs		.38	.50				.12
Meat and Fish:							
Beef, lean		1.50	1.00				
Beef, ground							.17
Liver			.50			.50	
Meat concentrate	1.08						
Pork, lean			.50				
Salmon			.25			.50	
Short ribs							.33
Soup bone							.06
Flour and Cereals:							
Bread, whole wheat			2.00				.06
Corn meal, yellow		1.75	.50	5.00	1.30	2.50	.66
Cracked whole wheat			.25				
Flour, white, enriched		1.00	.25	1.00		1.00	.69
Flour, whole wheat	4.62	1.00					.42
Grits							.08
Oatmeal		.25	.25				.18
Rice			.25				
Fats:							
Butter			.50				
Lard			.25				.17
Margarine		.75		1.00	1.00	.50	.40
Salt pork		.50	.25	.50	.50	.25	.26
Suet							.03
Molasses		.63	.25	.63	.63	.63	.24
Sugar		.50	.75	.50	.25	.50	.41

[1] This mixture also includes .04 lb. NaCl and .04 lb. Ca_2CO_3. Stock diet calculated on basis of amounts necessary to give 2100 calories per day. Ingredients are thoroughly mixed.

[2] The method of preparation for these diets was as follows: (a) Cereals, milk, sugar, and about half the fats were made into a soft pudding and baked until firm. (One-fourth of milk allowances was omitted and fed separately in test diet 4 because the large amount of milk produced a mixture too thin for feeding.) (b) Vegetables, having similar textures and cooking times, were cooked together until tender, then mashed or pureed. (c) Meats were fried in drippings from the salt pork, then ground. (d) Bread was ground after being dried in an oven at a very low temperature. (e) Items, such as cheese, molasses, salmon, canned tomatoes, and peanut butter, were added to the final mixture. (f) The entire mixture was placed in a Hobart mixer and beaten until as uniform as possible.

Sampling for the various assays was done by separating a batch (amount specified in market orders) of the food mixture into four parts and taking an aliquot from each. These were mixed and definite amounts taken for each assay — 5 gm. for the microbiological vitamin assays, 5 gm. for thiamine, 20 gm. for mineral assays, and 20 gm. for moisture. From the total weight of the batch, the amount per day of the specific vitamins and minerals furnished by the food mixture was determined. Riboflavin, niacin, and pantothenic acid were determined by the microbiological methods of Snell and Strong ('41), Snell and Wright ('41), and Pennington et al. ('41), respectively. Thiamine was determined by the yeast fermentation method of Schultz, Atkin and Frey ('42). For mineral analyses the sample was digested and made up to definite volume and aliquots were used for the determination of calcium, phosphorus and iron. Calcium was determined by an A.O.A.C. macro-method ('35), phosphorus by an adaptation of a method suggested by Snell and published by Koenig and Johnson ('42 a), and iron by the spectro-photometric method of Koenig and Johnson ('42 b). Moisture was determined by drying a moist sample in an electric oven at 40°C. for 24 hours. Four different batches of each of the seven food mixtures were analyzed and the results (table 2) are the averages of these four analyses. Table 2 also gives the caloric value of the various food mixtures and the average daily nutritive value.

On the dry weight basis considerable difference in the nutritive value of the various food mixtures is apparent. Test diet 1 is definitely poorer in all nutrients than the other test diets. Test diets 2, 3, and 4 are not unlike in nutritive value except for the lower concentration of niacin in diet 4, nor are they unlike the two Carpenter and Stiebeling control diets (II and III). The moderate-cost Carpenter and Stiebeling ration (III) is somewhat better than the minimum-cost diet (II), but the stock ration is much superior to all the proposed human diets in all nutrients except thiamine.

TABLE 2

Calorie, vitamin, and mineral value of seven food mixtures.

DIETS	CALORIES[1]	THIAMINE	RIBOFLAVIN	NIACIN	PANTOTHENIC ACID	Ca	P	Fe[2]
Per gram of dry weight								
		μg.	*μg.*	*μg.*	*μg.*	*mg.*	*mg.*	*mg.*
Control I	4.21	2.93	7.50	44.4	22.7	4.40	6.95	0.1530
Control II	4.73	2.13	3.40	24.1	16.3	1.80	2.10	0.0300
Control III	4.74	3.12	5.30	36.7	20.7	1.84	3.81	0.0630
Test 1	4.38	1.88	2.10	13.1	8.7	1.20	2.60	0.0330
Test 2	5.10	2.60	3.98	28.1	15.5	2.36	4.04	0.0682
Test 3	4.14	2.42	4.30	28.2	17.4	2.00	4.14	0.0435
Test 4	5.30	2.81	4.13	20.0	12.5	3.17	4.60	0.0536
Average daily values								
		mg.	*mg.*	*mg.*	*mg.*	*gm.*	*gm.*	*gm.*
Control I[3]	2100	1.46	3.75	22.20	11.30	2.20	3.47	0.0760
Control II	2600	1.17	1.87	13.25	8.97	0.99	1.15	0.0165
Control III	2700	1.78	3.02	20.91	11.80	1.05	2.17	0.0360
Test 1	3000	1.28	1.44	8.97	5.95	0.82	1.78	0.0226
Test 2	2102	1.07	1.64	11.57	6.38	0.97	1.66	0.0281
Test 3	2113	1.23	2.19	14.38	8.67	1.02	2.11	0.0222
Test 4	2529	1.35	1.98	9.60	6.00	1.52	2.20	0.0257
Recommended Sedentary woman	2100	1.20	1.80	12.00	....	0.80	1.32	0.0120
Moderately active man	3000	1.80	2.70	18.00	....	0.80	1.32	0.0120

[1] Values for control diets I and II and test diet 1 are from bomb-calorimeter experiments. The other values are calculated.

[2] Inaccurate because no care taken to prevent contamination. Useful only for comparative purposes.

[3] Not for human consumption. Given for comparative purposes.

Since the nutritive value of a food mixture depends upon the amount consumed, a better comparison of the food mixtures is obtained by consulting the figures on average daily values. A study of these shows that all of the proposed food mixtures are adequate in minerals, but that there are slight deficiencies in vitamin intake. Test diet 1, which, according to calculation was adequate on a 3,000 calorie intake, shows rather drastic vitamin deficiencies as actually assayed, for it

furnishes approximately half of the niacin and riboflavin, and three-fourths of the thiamine recommended as adequate. Of the other test diets, no. 2 is slightly low in thiamine and riboflavin, and no. 4 is low in niacin. The control diets proposed for human consumption are adequate except for a very slight thiamine deficiency in the minimum-cost diet. The rat stock diet, calculated to a 2100 calorie intake for comparison, is superior to all of the proposed food mixtures. At the time the calculations were made (1940–1941), control diet II (minimum-cost, adequate) cost approximately twice as much as any of the test diets. Of the test diets, no. 4 cost about 33% more than no. 3 and 17% more than nos. 1 or 2. Actual analyses show that three of the test diets compare favorably with one of the control rations, furnishing adequate amounts of mineral and approximately adequate amounts of three of the B-complex vitamins. According to calculations the test diets are also adequate in vitamin A and ascorbic acid. These findings indicate the possibility of providing adequate nutrition for approximately half of the amount necessary to purchase the minimum-cost adequate diet of Carpenter and Stiebeling. On this low expenditure level menus would be more monotonous and less pleasing than on the higher level, but probably less monotonous and unpalatable than those actually consumed at the present time. On the basis of assay results, the food mixtures represented by test diets 2, 3, and 4 may, with some degree of assurance, be recommended as insuring adequate nutrition for a sedentary woman.

RESULTS OF ANIMAL FEEDING EXPERIMENTS

As has already been stated, each of the seven food mixtures was fed to a group of young male albino rats and growth and food intake responses noted. The preparation of the mixtures for feeding was the same as for assaying. For the first series of experiments the food mixtures were made up every 6 days and stored in tightly capped fruit jars at 10°C.[4] These were

[4] Control diet II and test diet 1 were not fed as fresh mixtures.

taken from the refrigerator for only a brief period to remove a portion for daily feeding. Food was allowed to remain in the food cups only between 5:00 P.M. and 8:00 A.M., in an effort to prevent spoilage. This was not entirely successful, and the spoilage of food introduced a source of error that was impossible to evaluate. For this reason the feeding experiments were repeated, using dried mixtures. The various mixtures were prepared as before except that an equivalent amount of dried milk was substituted for fresh milk. After mixing, the diets were spread on paper and placed on trays in a current of hot air at a temperature of 65°C. for a period of 10 hours. Vitamin losses accompanying the drying were calculated by assaying samples from the batches before and after drying, and compensated for by adding equivalent amounts of the pure substances. Results of the rat-growth experiment are given in table 3.

TABLE 3

Average daily food intake and gain in weight of rats fed food mixtures.

DIETS	MOIST MIXTURES				DRIED MIXTURES			
	Number rats	Food intake[1]	Calories	Weight gain	Number rats	Food intake	Calories	Weight gain
		gm.		*gm.*		*gm.*		*gm.*
Control I	3	12.1	51	5.2	6	13.8	58	5.3
Control II[2]	..	...	..	..	5	11.1	53	4.0
Control III	3	10.8	52	4.3	5	10.9	52	4.5
Test 1[2]	..	...	..	..	6	9.7	42	2.2
Test 2	3	6.2	27	1.9	6	8.0	34	2.4
Test 3	3	9.8	44	3.6	4	11.2	50	3.8
Test 4	3	9.5	53	4.3	4	10.4	58	3.6

[1] Calculated to dry weight basis.

[2] Not fed in the moist form.

This table shows that, in general, the growth response and food intake of the experimental animals varied directly with the nutritive value of the diets. Control diet I was consumed in somewhat larger amounts and produced definitely better growth than any of the other diets. Control diet III, which had a somewhat higher nutritive value than II, produced

better growth, but the food intake of each was essentially the same. The lowest food consumption occurred on test diet 1 which was the poorest in nutritive value and produced the least growth. The only exception to the direct relationship between nutritive value and growth response was in test diet 2, a mixture similar to test diets 3 and 4 in nutritive value, but having a much lower food consumption and producing slower growth. Growth response and food intake on this diet were almost identical with those obtained on test diet 1, which was very much poorer in nutritive value. This result is probably accounted for by a difference in some dietary essential for which no assay was made, possibly in the biological value of the protein. This explanation is borne out by the fact that in an effort to find the limiting factors in test diet 1, which involved feeding the diet with many different supplements, only a supplement of meat concentrate produced a growth response essentially the same as that obtained with control diet I.[5]

The results of the animal feeding experiments seem to demonstrate the adequacy of the low-cost food mixtures represented by test diets 3 and 4. Advance in food prices has made these mixtures more expensive than at the time they were planned, but they are still cheap in comparison with control diets II and III.

SUMMARY

In an effort to find a combination of very cheap foods that might be used as a basis for the dietary in low-income groups, four food combinations, which had been calculated for adequacy and for which menus had been prepared, were assayed for certain vitamins and minerals; their adequacy for the rat was also tested. Two higher-cost food mixtures, designed for adequacy according to the suggestions of Carpenter and Stiebeling, and a stock ration for rats were similarly assayed and fed. Results showed that two of the low-cost food mixtures were similar to the higher-priced mixtures in their vita-

[5] Unpublished data from M. S. Thesis; Koenig, Ruth A., The Nutritive Value of Low-Cost Mixtures, Department of Home Economics, University of Texas, 1942.

min and mineral contents and in the growth response produced in animals. The cheaper food mixtures cost approximately half as much as the higher-priced ones.

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DIGESTIBILITY OF SOME ANIMAL AND VEGETABLE FATS

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In view of the world shortage of many kinds of food fats, further information concerning the nutritive properties of certain fats should be of interest. This paper contains the results of experiments with albino rats to determine the digestibility of the following: butterfat, beef fat (oleo stock), mutton tallow, cacao butter, corn oil, soybean oil, and coconut oil.

Information concerning the digestibility of several of these fats has been reported by others. Langworthy and Holmes ('15, '17) and Holmes (18), in experiments with human subjects, obtained the following digestive coefficients: butterfat 97, beef kidney fat 88, coconut oil 97.9, cocoa butter 94.9, corn oil 96.8, and soybean oil 97.5%. Rockwood and Sivickes ('18), found corn oil and cottonseed oil to be approximately 99% digestible in dogs. Holt et al. ('35) obtained the following digestive coefficients in normal infants: corn oil 96.9, soybean oil 93.7, coconut oil 88.7, butter 88.9, and a mixture of butter and corn oil 90.6%.

MATERIAL AND METHODS

The two lots of butter used were manufactured from sweet cream by the Bureau of Dairy Industry, United States Department of Agriculture, at Beltsville, Maryland. The second lot was obtained about 8 months later than the first. The butter was melted on a steam bath, filtered, and the fat was stored in completely-filled, tightly-covered glass jars.

Oleo stock (beef fat rendered at about 68°C.) and mutton tallow were obtained from large meat-packing establishments. Cacao butter (cocoa butter) was purchased from a wholesale drug house. The product was labeled U.S.P. Coconut oil and soybean oil were purchased from a manufacturer of oleomargarine. Corn oil in 1-pint tin cans was purchased on the open market. All the fats were stored in tightly-covered containers at about 4°C.

The formulas of the diets are shown in table 1. The casein was extracted with hot ethyl alcohol until practically free from lipids. Dextrose (U.S.P.) was used. The water soluble

TABLE 1

Formulas for the three diets fed to rats.

DIET NO.	FAT IN DIET	CASEIN	SALTS	DEXTROSE	YEAST VITAMINS	COD LIVER OIL CONCENTRATE [1]
	%	%	%	%	%	*cc./kilo. of diet*
1	None	18.6	4.0	75.4	2.0	100
2	5	20.0	4.3	68.7	2.0	100
3	15	23.0	5.0	54.5	2.5	115

[1] The saponified cod liver oil was extracted with ethyl ether and the ether extract washed with water until neutral. This concentrate contained the equivalent of 20 gm. of cod liver oil in 100 cc. After the addition of 100 cc. of cod liver oil concentrate to 1000 gm. of diet, the mixture was agitated by hand until the odor of ether had practically disappeared. The diet was then transferred to 1-quart glass jars which were sealed and placed in a refrigerator at about 6°C.

vitamins were supplied in the form of a commercial concentrate prepared from brewers' yeast. The label indicated that this product contained 240 B_1 and 60 B_2 Sherman units per gram. Vitamins A and D were supplied in the form of a concentrate prepared from saponified cod liver oil (see footnote to table 1). The salt mixture has been previously described by the writers ('40). Each diet was made up in the quantity of 1000 gm. In the diets containing 5 and 15% of fat, this foodstuff supplied 12.5 and 32.7% of the total energy value. The proportions of casein, salts, and yeast vitamins were adjusted so as to have approximately constant relationship to the

total energy value of each diet. The following factors were used in calculating the energy values: protein 4, carbohydrates (dextrose) 3.7, and fat 9 cal. per gram.

EXPERIMENTAL PROCEDURE

The melting point was determined by the capillary tube method and the iodine number by the Wijs method. The thiocyanogen number was determined in general according to the procedure outlined in Methods of Analysis, A.O.A.C. ('40), except that the thiocyanogen solution was made up to be approximately 0.2 N. An excess of at least 150% of the reagent was used and absorption was conducted for 24 hours in the dark at 19–20°C. Analyses were made in duplicate. The percentages of linoleic, oleic, saturated, and unsaturated fatty acids were calculated from the iodine and thiocyanogen numbers by the formulas given by Matthews et al. (p. 186, '41). The possible presence of small proportions of fatty acids more unsaturated than linoleic acid was disregarded.

Each diet containing either 5 or 15% of fat was fed to eight male albino rats for 60 days. At the beginning of the experiments the rats averaged 40 gm. in weight and their ages were 20 to 24 days, at most 28 days. The rats from different litters were distributed as evenly as practicable among the different groups. Each rat was kept in an individual cage provided with a raised screen bottom, a drinking vessel, and a self-feeder. The bottom of the cage was covered with a circular sheet of blotting paper which was changed as often as necessary. The rats were weighed twice weekly and a record was kept of feed consumed. The temperature of the rat laboratory was maintained at approximately 24°C. throughout the experiments.

A digestion test was conducted for 7 days with each rat after the experiment had been in progress about 50 days. The feed consumed during this test was weighed and all the feces excreted during the same period were dried to constant weight at 100°C. Visible hair was removed by a gentle blast of air.

The quantity of crude fat excreted was determined as follows: Approximately 2 gm. of dry feces was weighed into a 200-cc. Erlenmeyer flask, and 25 cc. of 30% potassium hydroxide and 50 cc. of 95% ethyl alcohol then added. The mixture was heated on a steam bath until saponification was complete and most of the alcohol had evaporated. The contents of the flask were transferred to a 500-cc. separatory funnel with water sufficient to make about 250 cc. An appreciable excess of concentrated hydrochloric acid was added and the funnel was shaken vigorously. When cool, the contents of the funnel were extracted twice with 100-cc. portions of petroleum ether to remove crude free fatty acids. The ether extract was treated with water to remove hydrochloric acid and then transferred to a tared 300-cc. Erlenmeyer flask containing a small piece of alundum to prevent loss of extract when the flask was heated on a steam bath to remove the petroleum ether. The flask was heated to constant weight at 100°C. The quantity of crude glyceride was calculated by multiplying the quantity of crude fatty acids by 1.045.

Metabolic fat in the feces was determined as follows. At the end of the 60-day test, each rat was transferred to the fat-free diet. After a preliminary period of at least 2 days to permit excretion of the feces from the previous diet, the excreta were collected for 7 days, dried to constant weight at 100°C., and the crude fat content was determined. This was considered to be metabolic fat.

The true digestibility of each fat was calculated in the usual manner after making correction for the quantity of metabolic fat excreted during the digestion test. It was assumed that each rat excreted the same quantity of metabolic fat, in proportion to non-fatty dry matter, as when it was fed the fat-free diet. The digestive coefficient was calculated for each of the eight rats fed a particular fat at each level of intake, but only the average value is reported.

EXPERIMENTAL RESULTS

Information concerning the composition of the fats tested is shown in table 2. The relationship between the melting points of these fats and their digestibility is discussed later.

TABLE 2

Composition of fats used in feeding tests with rats.

LABORATORY NUMBER AND KIND OF FAT	MELTING POINT	IODINE NUMBER	THIOCYANOGEN NUMBER	FREE FATTY ACIDS	FATTY ACIDS AS PERCENTAGES OF TOTAL FATTY ACIDS			
					Saturated	Unsaturated	Oleic	Linoleic
	°C.			%	%	%	%	%
Butterfat (4102)	36	32.9	26.3	0.17	71.3	28.7	20.9	7.8 [1]
Butterfat (4117)	33	34.6	30.2	0.20	66.8	33.2	28.0	5.2 [1]
Oleo stock (4099)	48	39.5	34.4	0.50	62.2	37.8	31.8	6.0
Mutton tallow (4097)	47	43.2	35.7	0.26	60.9	39.1	30.2	8.9
Cacao butter (4107)	28	37.1	31.9	0.96	66.0	34.0	27.9	6.1
Soybean oil (4105)		127.8	78.1	0.02	17.5	82.5	23.7	58.8 [1]
Corn oil (4101)		128.5	77.2	0.03	18.6	81.4	20.7	60.7
Coconut oil (4104)	26	11.1	7.3	0.04	92.2	7.8	3.3	4.5

[1] Includes fatty acids more unsaturated than linoleic.

The results of the digestion tests are shown in table 3. Duplicate tests were conducted at different times with butterfat no. 4102, mutton tallow no. 4097, and soybean oil no. 4105 at each level of intake in order to check the accuracy of the procedure. In each case the duplicate digestive coefficients agreed very closely. Soybean oil, corn oil, and coconut oil were highly digestible at each level of fat intake, followed by butterfat, oleo stock, mutton tallow and cacao butter. When the diets contained 15% of fat butterfat was somewhat more digestible, and oleo stock, mutton tallow and cacao butter were much more digestible. The reason for this difference is not apparent since correction was always made for metabolic fat.

Attention is directed to the lack of a definite relationship between the melting point of a fat and its digestibility, as may be seen by comparing the data in tables 2 and 3. For example,

mutton tallow melted at 47°C. and cacao butter at 28°, but mutton tallow had a considerably higher digestive coefficient when the diets contained 5% of fat, and an appreciably higher value when the diets contained 15%. Again, butterfat, cacao butter, and coconut oil, each melting below body temperature (37.6°C.), differed widely in digestibility. It is apparent that

TABLE 3

Average digestibility of some animal and vegetable fats in 7-day tests with male albino rats.

FAT USED	LABORATORY NO.	TRUE DIGESTIBILITY [1]	
		When diet contained 5% of fat	When diet contained 15% of fat
		%	%
Butterfat (1st test)	4102	87.5	90.7
Butterfat (2nd test)	4102	89.6	91.2
Butterfat	4117	87.9	90.2
Oleo stock	4099	74.0	86.7
Mutton tallow (1st test)	4097	74.2	85.0
Mutton tallow (2nd test)	4097	75.0	84.5
Cacao butter	4107	63.3	81.6
Soybean oil (1st test)	4105	98.7	98.5
Soybean oil (2nd test)	4105	98.3	98.1
Corn oil	4101	97.5	98.3
Coconut oil	4104	98.9	96.5
Minimum significant difference (odds 19–1)		3.8	1.7
Minimum significant difference (odds 99–1)		5.3	2.4

[1] Acknowledgment is made to Bradford Knapp, Jr., of the Animal Husbandry Division of the Bureau of Animal Industry, United States Department of Agriculture, for a statistical analysis of the experimental data.

some factor other than the melting point determined the relative digestibility of these fats, possibly their stearic acid content; pure stearic acid has a very low digestive coefficient. Although mutton tallow had a much higher melting point than cacao butter, the latter probably contained as high or a higher percentage of stearic acid (Lea, '38). The relatively low melting point of cacao butter is due to the even distribution of the saturated and unsaturated fatty acids in the fat molecules,

whereas mutton tallow contains a considerable proportion of fully saturated glycerides containing chiefly stearic and palmitic acids.

SUMMARY

The true digestive coefficients of several animal and vegetable fats were determined by experiments with young male albino rats. Each fat was incorporated in an otherwise adequate diet in the proportions of 5 and 15% by weight, corresponding to 12.5 and 32.7% of the total energy values of the diets.

When the diets contained 5% of fat, the following digestive coefficients were obtained: coconut oil 98.9, soybean oil 98.5, corn oil 97.5, butterfat 88.3, mutton tallow 74.6, oleo stock 74, and cacao butter 63.3%.

When the diets contained 15% of fat, the digestive coefficients were as follows: soybean oil 98.3, corn oil 98.3, coconut oil 96.5, butterfat 90.7, oleo stock 86.7, mutton tallow 84.8, and cacao butter 81.6%.

There was no consistent relationship between the melting points of the fats and their digestive coefficients.

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THE AVAILABILITY OF THE CALCIUM OF SOME NEW ZEALAND VEGETABLES

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Although work on the availability of calcium was started a considerable number of years ago (McClugage and Mendel, '18; Rose, '20), this problem has not had as much attention since then as might have been expected. This was largely due to the fact that the technique of balance experiments has only been perfected of recent years. In the early experiments the necessity for the subjects being in negative balance was not realized. Therefore the results obtained have sometimes been contradicted by later work (Kramer et al., '28, and Kinsman et al., '39). The use of standardized methods on rats in more recent years apears to have produced more consistent results.

Milk has been shown by numerous workers to be at least equal, and usually superior, as a source of calcium to any vegetable so far tested (Fincke and Sherman, '35; Fairbanks and Mitchell, '38; Kao et al., '38; Speirs, '39; Shields et al., '40; Fincke, '41). By the same studies it has been shown that different vegetables can vary considerably in the availability of their calcium as compared with milk.

Although it has been shown by Storms ('27) and Gregory ('28) that the calcium content of New Zealand vegetables may vary considerably from the values found on analysis of the same foods in other countries, there is no reason to believe that the availability of the calcium will necessarily vary to such an extent or pass outside the range of the probable error

of the experiment. Therefore vegetables were chosen which are widely used in New Zealand and which did not appear to have been studied elsewhere at the commencement of these experiments.

EXPERIMENTAL

The accretions of calcium from a test food, fed as the chief source of calcium in suboptimal amounts to young growing rats, were found by analysis and compared with those from skim milk powder which was chosen as the standard.

1. The paired feeding method was used on rats weighing approximately 40 gm. To save time and animals, trios were used, two vegetables being tested at a time. Litter-mate controls were sacrificed for analysis. The average calcium contents of thirteen males and twelve females were 0.62 and 0.65%, respectively.

2. Trio mates were fed equal restricted amounts of basal diet of the following composition (Shields et al., '40): dried ether-extracted beef, 30%; sucrose, 10%; lard, 10%; dried yeast, 10.5%; salt mixture (Ca and P free), 4.5%; powdered agar-agar (in experiment II only), 2%; starch to make up deficit, and cod liver oil as supplement, 2 drops per day.

The calcium content of the basal diet was kept low in order that as large a proportion of calcium as possible would be supplied by the test supplements — in these experiments 85%. The agar was included in the diet for the second experiment to give increased firmness to the feces so that they would fall through the screens more easily and lessen the likelihood of coprophagy.

3. Test supplements were fed to trio mates in amounts to provide the same weight of calcium but less than that needed for maximal calcification. The vegetables tested were silver beet greens and swede turnip in experiment I, and Savoy cabbage and parsnip in experiment II. It was thought that the silver beet used was the same as or very similar to chard. Only the edible portion of the vegetables was used and it was fed raw and fresh.

4. The basal diet and test supplements were analyzed for calcium. The average calcium content of the basal diet without agar was 0.021%; with agar, it was 0.027%. The figures for the calcium content of the supplements are contained in table 1.

TABLE 1

Average calcium content per cent of supplements and comparison with other figures.

	EXPERIMENTAL VALUE	PREVIOUS NEW ZEALAND FIGURES	ROSE ('39)	McCANCE AND WIDDOWSON ('40)
Silver beet greens	0.084 (5)[1]		0.10	
Swede turnip (rutabaga)	0.043 (5)	0.046[2] (4)	0.074	0.056
Cabbage	0.10[3] (10)	0.051[4] (10)	0.045	0.053[5]
Parsnip	0.040 (8)		0.060	0.055
Skim milk powder	1.34 (8)			

[1] The figures in brackets denote the number of determinations.
[2] Storms ('27).
[3] Savoy cabbage.
[4] Gregory ('28).
[5] Savoy cabbage boiled.

5. Gains in weight within the trios were kept as nearly equal as possible by giving sugar in concentrated aqueous solution to the laggard rats.

6. Feeding was continued until the rats in each trio gained 90–100 gm. body weight or had been on the experiment for 10 weeks.

7. The rats were killed and their bodies were analyzed for calcium after removal of the gastrointestinal tract. The figure so obtained minus the figure for calcium in the body at the beginning of the experiment (calculated from data from littermate controls) gives the calcium retention.

The method of analysis of calcium used throughout the experiments was a modification of the standard McCrudden ('11) method.

RESULTS

The average results for the two experiments are summarized in table 2.

TABLE 2

Results of two experiments comparing the availability of the calcium of vegetables with that of skim milk powder.

	EXPERIMENT I			EXPERIMENT II		
	Milk	Beet greens	Swede turnip	Milk	Savoy cabbage	Parsnip
Number of animals	10	8	10	10	8	9
Daily gain (gm.)	1.10	1.08	1.09	0.94	0.99	1.15
Ash content of carcass (gm.)	3.2	2.4	3.0	3.3	3.2	3.2
Ash content of carcass (%)	2.8	2.1	2.6	3.1	3.0	2.7
Ca content of carcass (gm.)	0.87	0.57	0.79	0.859	0.817	0.787
Ca content of carcass (%)	0.75	0.49	0.69	0.82	0.77	0.67
Retained Ca (gm.)	0.60	0.29	0.53	0.577	0.529	0.498
Ca intake (gm.)	0.75	0.75	0.75	0.671	0.657	0.654
Per cent retention of Ca	80	37	70	86	80	76
Availability of Ca, that of milk being taken as 100	100	46	87	100	93	88

The final figures, with probable errors, for the availability of the calcium of the vegetables, compared with that for skim milk powder taken as 100, are: Savoy cabbage 93 ± 2.8; parsnip 88 ± 2.5; swede turnip 87 ± 3.3; silver beet greens 46 ± 6.6.

The cabbage, parsnip and swede turnip are all very well utilized. The difference between cabbage and parsnip is on the borderline of significance but more data would be required to clearly differentiate between them. The difference between parsnip and swede turnip as sources of dietary calcium is negligible.

It appears that the calcium of silver beet greens is poorly utilized and it is noteworthy that this result was accompanied by the largest probable error. This variability may be partly due to coprophagy. It may also be due to variation in the individual ability to utilize calcium, which is probably accentuated at this low level of intake.

The low value given by silver beet greens is not surprising since Sherman ('41) states that "oxalates occur in very considerable quantities in certain plants, including beet greens, spinach and sorrel." Since silver beet is a variety of ordinary

beetroot, the leaves of which are referred to by Sherman, this vegetable would be expected to contain considerable quantities of oxalates also.

SUMMARY AND CONCLUSIONS

In a series of two experiments on growing rats, involving equalization of calcium intakes and, as far as possible, of gains in body weight among comparable animals, the calcium retention was determined by carcass analysis after a period of experimental feeding averaging 9 weeks. The availability of the calcium of various vegetables was compared in each case with that of the calcium of skim milk powder at levels of calcium intake insufficient to promote maximum calcification of the bones.

From the results obtained the following conclusions are drawn.

1. The calcium of milk is better utilized than the calcium of the vegetables tested.

2. The Savoy cabbage tested is an excellent source of calcium since its content of this element is very high; under the conditions of these experiments, this calcium was 93% as available as that of milk.

3. The calcium of swede turnips and parsnips is very well utilized. Under the conditions of these experiments their calcium was 87% and 88%, respectively, as available as that of milk.

4. Silver beet greens are a poor source of calcium in the diet, comparable in value to spinach since, under the conditions of these experiments, their calcium was only 46% as available as that of milk.

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